COGNITIVE VARIATION IN THREESPINED STICKLEBACK (*GASTEROSTEUS ACULEATUS)*

BY

MILES KUILING BENSKY

DISSERTATION

Submitted in partial fulfillment of the requirements
for the degree of Doctor of Philosophy in Ecology, Evolution, and Conservation Biology
in the Graduate College of the
University of Illinois at Urbana-Champaign, 2019

Urbana, Illinois

Doctoral Committee:

Professor Alison M. Bell, Chair and Director of Research
Professor Becky C. Fuller
Associate Professor Katy D. Heath
Assistant Professor Anna V. Kukekova

ABSTRACT

Cognition encompasses important mechanisms with which animals are able to adjust their behavior in response to environmental cues. These cognitive processes play a clear role in many fitness-related behaviors such as foraging, predator avoidance, and courtship. Thus, how these processes have evolved are of key scientific interest. Historically, research on the evolution of cognitive traits has largely focused on variation between species. However, particularly in the last couple of decades, there has been increasing interest in examining variation in cognition within a species. These studies no longer look at intraspecific variation as noise, but see it as being potentially adaptive and therefore impacting evolutionary trajectories. Yet, while many of these studies seemingly demonstrate the benefits of different cognitive traits, this has inevitably led to questions about why intraspecific variation is maintained. How costly are these traits? Are there trade-offs that maintain variation?

The aim of the research in this dissertation is to study the mechanisms that drive and maintain intraspecific variation in cognition in threespined sticklebacks. This is done from four different angles. First, I tested the hypothesis that learning is part of an overall suite of correlated traits related to how an individual copes with changes in the environment, and that trade-offs between early learning and responsiveness to changes in learning outcomes may maintain cognitive variation. I found that individuals that showed a higher cortisol stress response and that were more reactive to a predatory threat were slower to learn a novel discrimination task, but not necessarily faster to respond when learning conditions changed. Second, I tested the hypothesis that sticklebacks from populations inhabiting different environments are primed to learn different

cue associations faster within novel learning conditions. When individuals from two separate populations were trained on either a color vs. spatial discrimination task, the two populations excelled on different tasks: fish from a river habitat performed significantly better on the side version than they did on the color version, while the opposite was observed in fish from a pond habitat. Third, I explored the underlying causes of why some individuals are more responsive than others when there is a change in learning outcomes (i.e., differences in behavioral flexibility) by asking whether individual differences in reversal learning performance were more strongly associated with variation in boldness, neophobia and/or inhibitory control. I found that early performance on reversal learning trials was associated with all three behavioral traits, while time to criterion during reversal learning was independent of the other behaviors. Finally, I took advantage of the radiation of sticklebacks to ask whether behaviors predicted to facilitate adaptation to new environments (i.e., neophilia and inhibitory control) have evolved as stickleback have repeatedly colonized freshwater environments . I found heritable population-level variation in both behaviors, suggesting that increased flexibility has evolved during the stickleback radiation. Altogether these studies highlight the wide range of both intra- and inter-population cognitive variation that can be found in threespined stickleback and further elucidate how trait correlations and ecological differences may drive the maintenance of this variation.

ACKNOWLEDGEMENTS

First, I would like to thank Dr. Alison Bell for all of her support and mentorship over the years. I appreciated her patience as I tried to find my own niche in the lab, and her support as my family went through a number of milestones and challenges.

I would like to thank all the people who helped support my interests in research and animal behavior that got me to the University of Illinois. Thank you to Dr. Michael Beecher for giving me my first opportunity to participate in research as a part of his lab. A huge thank you to Dr. James and Dr. Renee Ha for their years of mentorship and friendship, particularly during those times when I was unsure of which to path to go down. Thank you to Dr. Sam Gosling and Dr. David Sinn for your mentorship during my time at the University of Texas at Austin, and for your continued support when I decided that I wanted to move in a different direction.

From my time at the University of Illinois, I have been fortunate to interact with some amazing professors and staff, including all the member of my committee. I have really appreciated your guidance, and the opportunity to learn from and teach with you. I have also been extremely fortunate to meet many friends, and I would particularly like to thank all the members of the Bell Lab for their support, assistance, and friendship over the last 6 years.

I would like to give special thanks to my family. There is no way I would have been able to accomplish this goal without their support. My parents have always supported my interests, no matter where they took me, and I could not ask for more as a son.

Finally, I would like to thank my wife, Jessie. Her constant love and support over the last 20 years have meant everything to me and have helped me stay grounded. I am eternally grateful.

This dissertation is dedicated to my girls, Jessie, Leona, and Mabel

TABLE OF CONTENTS

CHAPTER 1: TESTING THE PREDICTIONS OF COPING STYLES THEORY IN THREESPINED STICKLEBACKS[1]

ABSTRACT

Coping styles theory provides a framework for understanding individual variation in how animals respond to environmental change and predicts how individual differences in stress responsiveness and behavior might relate to cognitive differences. According to coping styles theory, proactive individuals are bolder, less reactive to stressors, and more routinized than their reactive counterparts. A key tenet of coping styles theory is that variation in coping styles is maintained by tradeoffs with behavioral flexibility: proactive individuals excel in stable environments while more flexible, reactive individuals perform better in variable environments. Here, we assess evidence for coping styles within a natural population of threespined sticklebacks (*Gasterosteus aculeatus*). We developed a criterion-based learning paradigm to evaluate individual variation in acquisition and reversal learning. We observed strong individual differences in boldness, cortisol production, and learning performance. Consistent with coping styles, fish that released more cortisol were more timid in response to a predator attack and slower to learn a color discrimination task. However, there was no evidence that reactive individuals performed better when the environment changed (when the rewarded color was

[1] Chapter 1 was published in its entirety by Behavioural Processes. Bensky, M. K., Paitz, R., Pereira, L., Bell, A. M. (2017). Testing the predictions of coping styles theory in threespined sticklebacks. Behavioural Processes 136: 1-10. This article is reprinted with permission of the publisher and is available from https://doi.org/10.1016/j.beproc.2016.12.011

reversed). The failure to detect trade-offs between behavioral routinization and flexibility prompts other explanations for the maintenance of differing coping styles.

INTRODUCTION

Interest in individual variation in animal cognition is booming (e.g., Bensky et al. 2013; Chittka et al. 2009; Matzel et al. 2003; Niemela et al. 2013; Thornton and Lukas 2012). For example, a growing number of studies are showing that there is substantial intra-specific variation in performance on different cognitive tasks in a wide range of taxa (e.g., birds: Guillette et al. 2015; dogs: Nippak and Milgram 2005; primates: Herrmann et al. 2009; rodents: Galsworthy et al. 2002). Additionally, there has been increased interest in how individual variation in cognition may correspond with consistent individual differences in behavior, or animal personalities (Budaev and Brown 2011; Carere and Locurto 2011; Locurto 2007; Sih and Del Giudice 2012). As a result, studies exploring the nature of the correlations between various animal personality axes and cognitive differences are on the rise (e.g., Bebus et al. 2016; Ruiz-Gomez et al. 2011; Sneddon 2003), and more attention is being called to developing best-practices for examining cognitive differences to address these types of questions (Griffen et al. 2015). However, the proximate and ultimate causes of correlations between cognition and behavior are presently unknown.

Coping styles theory offers a framework for understanding individual variation in cognition and behavior. According to this theory, variation in cognition is a part of a suite of correlated traits adapted for coping with changes in the environment, with individuals varying along the proactive-reactive axis (Koolhaas et al. 1999). At one end of a continuous distribution

are proactive individuals, which are relatively exploratory, bold, and aggressive compared to their reactive counterparts at the other extreme. A key tenet of coping styles theory is that a cost of being proactive is reduced behavioral flexibility: proactive individuals establish initial learning contingencies quickly, largely due to their exploratory nature, but then become routinized and perform poorly when the reward is reversed (Koolhaas et al. 1999). Reactive individuals, on the other hand, are more stress reactive and timid, and may be slow initial learners but then quickly respond to environmental change. Following this reasoning, it has been suggested that variation along the proactive-reactive axis is maintained by fluctuating selection pressures (Thornton and Lukas 2012). Specifically, proactive, inflexible individuals might have higher fitness in stable environments while reactive, flexible individuals may be better suited for environments that experience moderate change (Benus et al. 1990; Guillette et al. 2011; Niemela et al. 2013; Ruiz-Gomez et al. 2011; Verbeek et al. 1994). Additionally, such variation may be driven by frequency-dependent benefits (Wolf et al. 2008) or differences in dispersal states (Canestrelli et al. 2016).

Given that different coping styles are expected to reflect variation in how individuals respond to challenging conditions or stressors, one of the proximate mechanisms underlying coping styles is thought to involve the hypothalamic-pituitary-adrenal (HPA) axis (Koolhaas et al. 2010). Indeed, some studies suggest that proactive individuals have relatively low stress reactivity and higher sympathetic activity (Carere et al. 2010; Sorenson et al. 2013), but some authors have suggested that stress responsiveness is an independent axis of variation (Koolhaas et al. 2010). Upon perceiving a stimulus as stressful, the hypothalamus initiates a cascade that culminates in an increase in circulating glucocorticoid levels (Sapolsky et al. 2000). Once the stimulus is removed or no longer perceived as a threat, feedback loops are triggered to shut down

the production of glucocorticoids (Lupien et al. 2009). Several studies in diverse organisms have shown that individuals consistently vary in stress responsiveness (repeatability of glucocorticoid hormone levels: Boulton et al. 2015; Cockrem et al. 2009; Cook et al. 2012; Fürtbauer et al. 2015; Ouyang et al. 2011; selection on glucocorticoid responsiveness: Øverli et al. 2005; Pottinger and Carrick 1999; Roberts et al. 2007), therefore individual variation in the transient production of glucocorticoids has been hypothesized to underlie variation in the behavioral response to stress (Carere et al. 2010; Sorensen et al. 2013). These physiological mechanisms have also tied coping styles to potential fitness differences in terms of stress-related health and disease susceptibility (Constantini et al. 2012; De Boer et al. 2016).

Individual variation in stress responsiveness has also been linked to variation in cognitive performance (Lupien and McEwen 1997; Kosten et al. 2012). For example, lines of trout that had been selected for divergent post-stressor cortisol levels differed in cognitive performance: trout that produced high levels of cortisol in response to a stressor were quicker to alter their behavior in response to food relocation during reversal trials (Ruiz-Gomez et al. 2011). These reactive fish were also faster to stop a conditioned response during extinction trials (Moreira et al. 2004). Overall, there is a plethora of data (e.g. McEwen and Sapolsky 1995; Øverli et al. 2005, 2007; Schoech et al. 2011) demonstrating that glucocorticoids are related to behavioral responses to stress and cognitive performance. However very few studies have characterized HPA axis function, behavior, and cognition simultaneously, and those that have tend to mostly focus on selected lines or domesticated animals (e.g. Benus et al. 1990; Bolhuis et al. 2004; Øverli et al. 2002, but see Bebus et al. 2016; Øverli et al. 2007). Attempts to simultaneously characterize several different facets of coping styles in natural populations are rare due to the time intensive nature of collecting extensive individual-based data on many subjects.

Here, we took a high-resolution approach to test the generalizability of whether individual differences in cognitive performance are part of a larger suite of behaviors, or coping styles, in a natural population. Again, much of the work on coping styles has been focused on mammalian and avian species (Carere et al. 2010), and while significant work has also been done looking at fish, particularly salmonids (Øverli et al. 2005), we were interested in further expanding the research applied to these taxa with a focus on natural variation. Here we tested the threespined sticklebacks *(Gasterosteus aculeatus*), a species renowned for their natural intraspecific variation in behavior (e.g., Bell 2005; Dingemanse et al. 2007; Girvan and Braithwaite 1998; Huntingford 1976; Mamuneas et al. 2015; Odling-Smee and Braithwaite 2003; Stein and Bell 2012), and a system that was recently used to investigate behavioral and endocrine associations in coping with increased predatory threat (Fürtbauer et al. 2015). Additionally, this species has been established as being conducive to fish cognition studies (Girvan & Braithwaite 1998; Odling-Smee and Braithwaite 2003). We repeatedly measured stress reactivity and 'boldness' towards a model predator on the same individuals and then asked whether those traits were related to variation in cognitive performance. We developed a criterion-based method to quantify the distribution of individual variation in cognitive performance in a discrimination-reversal learning paradigm. Subjects were trained to associate a food reward with one of two colors. Then, after a success criterion was reached, the stimuli and reward association were reversed. We interpret performance on the reversal task as behavioral flexibility (as in Pintor et al. 2014; see Stamps 2015), where persistent (proactive, routinized) individuals continue to approach the previously learned cue while more flexible (reactive) individuals more quickly modify their behavior and learn the new association. According to coping styles theory, we predicted that: 1) bolder (proactive) individuals are less stress reactive; 2) bolder individuals

learn faster; 3) bolder individuals are less flexible; 4) individuals that are more stress reactive are more flexible.

MATERIALS & METHODS

Overview of the experiment

We measured stress reactivity, "boldness", learning and behavioral flexibility on the same individual sticklebacks in a fixed order. Individuals were measured for stress responsiveness on two occasions. We used a non-invasive method that samples cortisol released from the gills into the water following confinement stress for 4.5 hours (Sebire et al. 2007; Sebire et al. 2009; Aubin-Horth 2012). Individual differences in "boldness" were measured by testing the same individuals for their response to a model predator on four occasions. Finally, the same subjects were tested for learning performance in a color discrimination task and for behavioral flexibility in a reversal-learning task.

Subjects

Adult sticklebacks were collected from Putah Creek, CA in April 2014 prior to the onset of the breeding season. Fish were transported to the University of Illinois Urbana-Champaign where they were housed in 37 L tanks in groups of five for approximately four weeks until experiments began. Fish were housed in 20 degrees Celsius water and were placed on a summer (16L: 8D) photoperiod. A recirculating flow-through system consisting of a series of particulate, biological, and UV filters (Aquaneering, San Diego, USA) was used to clean the water. 10% of the water volume in the tanks was replaced each day. The fish were fed a mixed diet consisting

of frozen bloodworms, frozen brine shrimp, and frozen Mysis shrimp ad lib each day except during the learning trials (see below). Ten individuals were randomly selected for this experiment (females: n = 6, average length = 43.8 mm, average mass = 1.02 g; males: n = 4, average length = 42.5mm, average mass = 0.87g). Females were not gravid during the experiment and males showed no evidence of nuptial coloration. Each individual was marked by clipping a unique combination of their dorsal and/or pelvic spines.

Cortisol release rate

The purpose of this experiment was to determine whether there were consistent individual differences in stress reactivity. The hypothalamic-pituitary-interrenal (HPI) response of each fish was characterized by measuring the amount of cortisol released into water at five time points over the course of 4.5 hours. This process was repeated one week later in order to determine whether individual differences in cortisol release rate were stable over a one-week period.

Fish were transferred to individual static 100 mL tanks and left undisturbed for 30 minutes. Then, a 50 mL water sample was collected from a release valve at the bottom of the tank. The water supply was turned on and allowed to run for 30 minutes to flush the cortisol-laden water from the tank. Preliminary trials confirmed that turning on the water supply for 30 minutes flushed the tank sufficiently such that cortisol could no longer be detected in the water. Following the 30-minute flush, the water supply was turned back off to allow another 30 minutes of static water to collect cortisol. This process was repeated so that we generated water samples from minutes 0-30, 60-90, 120-150, 180-210, and 240-270. Fish were returned to their home tank once sample collection was complete.

Water samples (10 fish x 5 samples/trial x 2 trials = 100 samples) were frozen at -20°C until steroid quantification. Freeze storage of water samples does not influence steroid concentrations (Ellis et al. 2004). Steroids were extracted from water samples by pulling water through C18 Sep-Pak cartridges (Waters Ltd.) that had been primed with 5 mL methanol followed by 5 mL distilled water. After the sample had dripped through at a rate of approximately 2 mL/min, the cartridge was washed with 5 mL of distilled water and the steroids were then eluted with 5 mL of diethyl ether. The ether was dried under nitrogen and submitted to Metabolomics Center, Roy J. Carver Biotechnology Center, University of Illinois at Urbana-Champaign.

Samples were analyzed via mass spectrometry with the 5500 QTRAP LC/MS/MS system (AB Sciex, Foster City, CA). The 1200 series HPLC system (Agilent Technologies, Santa Clara, CA) includes a degasser, an autosampler, and a binary pump. The LC separation was performed on a Phenomenex C6 Phenyl column (2.0 x 100mm, 3µm.) with mobile phase A (0.1% formic acid in water) and mobile phase B (0.1% formic acid in acetonitrile). The flow rate was 0.25 mL/min. The linear gradient was as follows: 0-1 min, 80%A; 10 min, 65%A; 15 min, 50%A; 20 min, 40%A; 25 min, 30%A; 30 min, 20%A; 30.5-38 min, 80%A. The autosampler was set at 5°C. The injection volume was 5 µL. Mass spectra were acquired under positive electrospray ionization (ESI) with the ion spray voltage of 5500 V. The source temperature was 500 °C. The curtain gas, ion source gas 1, and ion source gas 2 were 36 psi, 50 psi, and 65 psi, respectively. Multiple reaction monitoring (MRM) was used to measure cortisol with the Q1-Q3 transition of 363.1 – 121.0 (m/z). In order to control for possible size effects, cortisol release rates were calculated as the amount of released cortisol per gram of body weight per hour (ng/g/hr).

Boldness assays

Three weeks after the steroid measures were complete, each individual was observed in a standardized behavioral assay designed to measure boldness in response to a predatory threat. The response of each individual fish to a model predator was recorded. Each individual was tested on four consecutive days in order to determine whether individual differences in behavior were consistent over time.

The testing arena was a circular plastic pool (150 cm diameter) filled to a depth of 10 cm of water (Figure 1.1a). An opaque refuge (10 cm diameter, 10 cm height) was placed in the center of the pool. The pool was divided into nine equally-sized sections (one centered, eight around the perimeter), with drawn lines demarking each of these sections. A rock was placed into each of the perimeter sections, providing objects to "explore" or additional refuges.

At the start of an assay, a randomly selected individual was gently caught from their home group tank, identified via spine clips, and then placed into the refuge at the center of the pool where it was allowed to acclimate for three minutes. Behavioral observations were recorded by an observer positioned behind a blind. After three minutes, the observer opened the side of the refuge remotely and waited for the fish to emerge. After fully emerging, the fish was given three minutes to freely explore the pool. Baseline activity levels were recorded over this time period by recording the number of transitions the fish made between sections (*sections before the attack*). We then simulated a predator attack by moving a clay predator model (sculpin, 20 cm length; Figure 1.1b) from a hidden location into the section of the pool that contained the focal fish. Sculpin (genus: *Cottus*) are a natural predator to freshwater populations of stickleback (Pressley, 1981). This stimulus elicited a freezing response, which has been found to be an effective antipredator response (McGhee et al. 2012). After the individual resumed movement,

we recorded the number of transitions made between sections for three more minutes (*sections after the attack*). To confirm that the predator attack influenced behavior, and that movement after the predator attack is a reasonable measure of boldness, we compared movement before and after the attack. Locomotion is conspicuous, therefore we predicted that on average the fish should move into fewer sections after the attack if they were exhibiting an antipredator response. After the completion of each assay the individual was placed back into their group tank. One individual died of unknown cause between day 3 and 4 of the behavioral assays; the behavior of this fish was not obviously different from the others. Therefore, the data from the deceased fish is included in all analyses except those involving the learning trials since those were carried out after the boldness assays.

Learning trials

Three weeks after the 'boldness' assays were complete, we measured individual differences in performance in an associative learning task. Each fish was simultaneously presented with a yellow and a blue cup, with the yellow cup rewarded with food. We infer that a fish learned the color association task when it reached criterion (two consecutive days of at least 8 out of 10 correct trials).

Each fish was moved into a 36 cm long x 33 cm wide x 24 cm high (26.5 L) tank where they were housed individually. Prior to the learning trials, fish were acclimated to eating out of cups, and to repeatedly being placed in a shelter. Additionally, through these pre-training trials the fish were given the opportunity to learn that food could potentially be available at two different food sites. The fish only obtained food during the learning trials in order to maintain motivation. All the fish readily ate food from both food sites over these pre-training sessions and

consistently decreased their latency to emerge from the shelter to approach one of these food sites, which we interpreted as motivation to access these sites. Criteria were used to assure that the fish were sufficiently acclimated to the training environment and motivated to access the food sites before training trials began on the following day. Specifically, individuals were required to eat out of both cups within 10 minutes on three consecutive days before moving on to the shelter acclimation phase. For this phase, individuals needed to emerge from the shelter, find the available food and eat it within 10 minutes on two daily trials for three consecutive days before moving on to the training trials.

Yellow/Blue Discrimination Phase: Fish received one learning session per day. Each session consisted of 10 trials. All of the nine fish completed a trial before the next round of trials was started (i.e., all fish received Trial 1 before moving onto Trial 2), so there was an average of 15-20 minutes between each trial for a single individual. The side that was rewarded during each trial was pseudo-randomized for each individual fish according to the following two rules: 1) half of the trials were rewarded on each side, and 2) a single side could not be rewarded more than two trials in a row. During each trial, the fish was placed in the shelter at the back of the tank. While the fish was in the shelter, two reward cups were placed as they were during the pre-training trials (Figure 1.1c). A petri dish was then placed into the base of each cup. One petri dish was designated the "Incorrect Reward Dish," which contained five bloodworms and was covered with electrical tape to prevent access to the worms. Holes in the tape allowed chemical/odor cues to emanate from the dish, but the holes were too small for the bloodworms to be removed. The other petri dish was designated the "Correct Reward Dish." This dish had electrical tape lining its rim to limit the odor cues from the tape being used as a discriminatory cue, but nothing obstructed access to the contents of the dish. As seen with many other visual

discrimination studies (e.g., Nippak and Milgram 2005; Ruiz-Gomez et al. 2011), making sure food cues were present in both potential food sites prevented the food cues themselves from directing the animal's choice. During this phase the yellow cup was always rewarded, so it was placed on the designated rewarded side and the "Correct Reward Dish" was placed in it. Using a dropper, two bloodworms were placed into the "Correct Reward Dish" per trial. The "Incorrect Reward Dish" was then placed into the Blue cup on the opposite side. After the cups were set up, and the fish had been in the shelter at least a minute, the cork of the shelter was removed. Once the fish had exited the shelter, we recorded whether the fish approached the designated choice region of the rewarded (yellow) or unrewarded (blue) cup (see Figure 1.1c). If the fish first approached the choice region of the yellow cup, the trial was marked as a "correct choice." If the fish first approached the choice region of the blue cup, the trial was marked as an "incorrect choice." Regardless of whether the first choice was correct, the fish was allowed to explore the tank until it found the "Correct Reward Dish," and was given a chance to eat the worms. This was important because it gave each individual an equal opportunity to associate the rewarded stimulus cues with the food reward, regardless of the first choice. If the fish entered at least one of the choice regions, but did not eat the worms within 5 minutes of making a choice, the trial ended, and it was noted that they did not receive food on that trial. The Yellow/Blue Discrimination phase continued until the fish had met the criterion of two consecutive days of at least 8 correct choices out of 10 trials. This criterion is similar to those used in other published discrimination learning studies (Guillette et al. 2011; Nippak et al. 2003; Tapp et al. 2003), though we decided to base our criterion exclusively on consecutive day performance in order to help ensure long-term retention of the trained contingencies. From a statistical standpoint, this criterion makes it very unlikely that a fish would perform at criterion level simply due to chance

(binomial distribution: $p = 0.003$). Once this criterion was met, the Reversal Learning Phase started during the next session.

Measuring individual differences in learning flexibility

The purpose of this experiment was to quantify individual differences in reversal learning performance, which we interpret as flexibility (Bond et al. 2007). This experiment was the same as the Yellow/Blue discrimination phase except that now the blue cup was rewarded during each trial. The criterion was again that the fish had to get 8 out of 10 trials correct on two consecutive days.

Statistical Analysis

Given previous findings showing correlations between boldness and sex (e.g., Brown et al. 2007; Harris et al. 2010) and body size (e.g., Brown and Braithewaite, 2004), we tested for differences between the sexes using t-tests, and we tested for an effect of body length on bold behavior using Pearson correlations.

To estimate repeatability, we used Bayesian statistics with Markov Chain Monte Carlo simulations using the MCMCglmm package (Hadfield, 2010) in R 3.1 (http://www.r-project.org/). We computed the repeatability of stress reactivity by computing the average cortisol across the five measures within a week, and estimated the repeatability of average cortisol across the two weeks. Average cortisol was normally distributed. Week and Individual were included as random effects. We estimated the repeatability of boldness (*sections after the attack*) across four days, while including Day and Individual as random effects and Sex as a fixed factor since there was evidence of a sex effect on behavior (see Results). As our measure of

boldness is a count-based measure, a Poisson distribution was used. For all analyses, we used non-informative proper priors (Hadfield 2010) with 500 000 iterations, thinning of 100 iterations and a burn-in of 1000 iterations. Through these simulations, 95% confidence intervals were generated and reported (in brackets) around our repeatability estimates. To determine the significance of our estimates we evaluated the lower bounds of the 95% confidence interval and looked to see that they were not near zero. To confirm that the fish were responding to the predator attacks, we compared the average sections entered before and after the attacks, and performed a t-test to test to see if there was significant decrease in movement after seeing the predator.

Finally, to test the predicted associations made by coping styles theory, we analyzed pairwise correlations between average cortisol release rate, sections after the attack, sessions to reach criterion on initial learning, and performance on the first reversal session using Pearson correlations. In the case of correlations involving boldness, since there was a trend for an effect of sex, we used partial correlations controlling for sex. All statistical analyses were carried out using R 3.1 (http://www.r-project.org/).

RESULTS

Cortisol release rate

On average, cortisol release rate declined over time within a session, such that cortisol release rate was lower four hours after isolation compared to initially ($t_9 = 2.37$, $p = 0.0417$, Figure 1.2a). Additionally, there were consistent individual differences in cortisol release rate over time, with some individuals consistently releasing more cortisol than others across the two

sampling periods (repeatability of sample average, R =0.6159 [0.2252, 0.9218], n=10, Figure 1.2b).

Boldness

There were consistent individual differences in boldness across the four measurements (R = 0.4980 [0.0817, 0.8244], n=10, Figure 1.3a).

On average, sticklebacks were less active after the simulated predator attack compared to before ($t_9 = 4.026$, p = 0.003; Figure 1.3b), which suggests that the fish perceived the model predator as a threat. There was no indication that the decline in activity reflects habituation to the testing environment: there was no decline in activity across the 3 minutes prior to the predator attack (1st minute = 11.23 sections, 2nd minute mean = 12 sections, 3rd minute mean = ll.95 sections). There was a trend for males to be more active than females after the attack ($t_8 = 2.248$, p=0.055; males: n=4, mean ± sd = 27.34 ± 7.599 sections; females: n=6, mean ± sd = 17.42 ± 6.343 sections). We did not detect a difference in body length between male and female fish ($t_7 = 0.472$, p=0.651; males: n=4, mean ± sd = 42.5 ± 4.203 mm; females: n=6, mean ± sd = 43.83 ± 4.622 mm), and did not detect a significant correlation between length and boldness ($t_8 = 0.6288$, p=0.547). Therefore, variation in activity among individuals or between males and females was not driven by differences in body size.

Learning

Sticklebacks learned to associate the yellow cup with food. Indeed, all individuals met the criterion (80% correct for two consecutive sessions) in the initial learning phase (Figure 1.4a). During the first few sessions, individuals appeared to choose randomly, but average

performance improved with subsequent training (Figure 1.4a). On average, it took 5.78 sessions (sd=2.86) to reach the criterion, but individuals differed: the fastest individual met the criterion in two sessions, while the slowest individual took eleven sessions to reach criterion.

Flexibility

It took longer for fish to learn to associate the blue cup with food after they had been trained to associate the yellow cup with food. Indeed, performance during the first session of reversal training was very poor: on average, sticklebacks went to blue, the previously-learned cue, during 79% of the trials in the first session, which suggests that sticklebacks were relying on a previously-learned association (Figure 1.4b). On average, it took 8.67 sessions (sd=3.77) to reach criterion during the reversal phase (Figure 1.4b). The fastest fish during this phase met the criterion in three sessions while the slowest learner never reached criterion, even after 17 sessions. Interestingly, the individual that took the longest to reach criterion during the initial learning phase also took the longest to reach criterion during the reversal learning phase.

Testing the predictions of coping styles theory

Coping styles theory predicts that bolder individuals are less physiologically responsive to stress. Consistent with this hypothesis, we found that bolder fish had a lower average cortisol release rates across the two weeks sampled (partial r controlling for sex = -0.707, n=10, p = 0.022, Figure 1.5a). We also found support for the prediction that bolder individuals learn faster: bolder fish reached criterion during in the color discrimination task in fewer trials than relatively shy fish (partial r controlling for sex = -0.712, n=9, p = 0.032, Figure 1.5b). Moreover, the

individuals that released more cortisol over time were also the ones that took more trials to reach criterion in the color discrimination task ($r = 0.722$, $n = 9$, $p = 0.028$, Figure 1.5c).

Coping styles theory also predicts that bold (proactive) individuals are more routinized and less behaviorally flexible. However, we did not detect any statistically significant relationships between the number of sessions to criterion during reversal training and any of the other measures, and indeed the direction of the observed relationships between traits is *opposite* to our predictions. For example, we predicted a negative relationship between initial color discrimination performance and reversal learning. However, sessions to initial criterion and sessions to reversal criterion were not negatively correlated, and indeed the (non-significant) pattern was positive ($r = 0.467$, $n = 9$, $p = 0.206$, Figure A.1a). Similarly, contrary to our predictions, a negative correlation was not observed between boldness and reversal learning, and indeed the (non-significant) pattern was that bolder individuals performed better during reversal learning (partial r controlling for sex = -0.491, $n = 9$, $p = 0.180$, Figure A.1b). Finally, again contrary to our original predictions, *we* observed more stress reactive individuals took longer to reach the reversal criterion, although this was not statistically significant ($r = 0.458$, $n = 9$, $p = 0.2155$, Figure A.1c).

It is possible that the number of mistakes during the first reversal session might be a better measure of behavioral flexibility than time to criterion (Bebus et al. 2016; Bond et al. 2007). For example, individual differences in time to reversal criterion might reflect a general difference in learning performance rate, whereas initial performance (number of mistakes) might more effectively capture how individuals immediately respond to a change in the environment. If this is the case, then proactive individuals might have made more mistakes during the first reversal session. However, we found no evidence that proactive individuals made more mistakes;

we did not detect any significant correlations between the number of mistakes and the other measures (time to criterion during initial learning ($r = 0.064$, $n = 9$, $p = 0.870$), cortisol (0.483, $n = 9$, $p = 0.188$), boldness (partial r controlling for sex = -0.080, $n = 9$, $p = 0.838$); Figure A.1 d-f).

DISCUSSION

We observed strong inter-individual variation among wild-caught individual sticklebacks from a natural population. These results are consistent with several studies that have documented extensive variation among individuals within stickleback populations (e.g. Bell 2005; Dingemanse et al. 2007; Fürtbauer et al. 2015; Huntingford 1976). For example, some sticklebacks were consistently more bold than others toward a model predator. Stable individual differences in boldness is consistent with previous studies showing dramatic inter-individual differences in boldness in this species (Bell 2005; Huntingford 1976; Mamuneas et al. 2015) and with the growing literature on personality in animals generally (Bell et al. 2009). Also consistent with past studies on fish boldness was the trend that males are bolder than females (e.g., Brown et al., 2007; Harris et al., 2010; King et al. 2013). Additionally, we also observed that some individual sticklebacks were consistently more stress reactive than others. This result agrees with previous studies in stickleback (Fürtbauer et al. 2015) and other animals showing intraspecific variation in the responsiveness of the hypothalamic-pituitary-adrenal axis to stressors (e.g. Boulton et al. 2015; Cook et al. 2012; Cockrem et al. 2009; Ouyang et al. 2011). Finally, some individual sticklebacks were also clearly faster at learning a color discrimination task than others, and there was substantial inter-individual variation in performance on a reversal learning task.

Examining the correlations of these facets, previous work on stickleback found basal cortisol and anti-predator behavior covaried within individuals across multiple trials (Fürtbauer et al. 2015). In the current study we found evidence that across individuals boldness covaried with stress reactivity and initial learning as predicted by coping styles theory, but we found no support for the prediction that timid, stress reactive individuals are more behaviorally flexible even after controlling for the effect of sex on boldness.

One of the strengths of this study is that our criterion-based learning protocol captured a wide swath of variation in performance on a cognitive task. While our approach required an extended pre-training period and prolonged testing of certain individuals (e.g. it took one individual 11 sessions to finally reach criterion during initial testing), all of the animals in this study eventually learned to associate the yellow cup with the food reward. Further evidence of the effectiveness of our training protocol is revealed by the animals' poor performance during the first session of reversal testing: whereas individuals appeared to choose randomly between the two colors during the first few sessions of initial testing, they showed a strong preference for yellow (the previously rewarded color) during the first few sessions of reversal testing (Figure 1.4b). Yet, again the fish were all eventually able to reach the reversal criterion, suggesting that the use of this paradigm was appropriate for this system.

Consistent with the predictions of coping styles theory and with previous studies (Hennessey et al. 2001; Raoult et al. 2012; Tudorache et al. 2013, but see Boulton et al. 2015; Ferrari et al. 2013), individual sticklebacks that were bolder were also less stress reactive. Moreover, less stress reactive individuals learned faster, and bolder individuals learned faster (similar to Bebus et al. 2016). While the effects of cortisol on learning and memory can be quite dynamic, these findings are consistent with previous research suggesting that long-term baseline

elevations in cortisol impede learning (Lupien et al. 1998; 2009). The positive association between boldness and cognitive performance is consistent with other studies (e.g. Dugatkin and Alfieri 2003; Guillette et al. 2009; Sneddon 2003; though see Cole et al. (2011)), and with previous work on trout that found that bold individuals were more behaviorally labile than their timid counterparts (Frost et al. 2007; Thomson et al. 2012). In some previous work, however, it is difficult to know whether bold individuals learned faster because they were more exploratory and had more opportunities to interact with their environments and therefore faster to learn (DePasquale et al. 2014; Sih and Del Giudice 2012). Importantly, we ran trials until the fish found the correct cup, even if the correct cup was not their first choice, which standardized the opportunity to gain information from each trial for all individuals in this study (Griffen et al. 2015). Therefore, bold individuals were not able to gather any more information that timid individuals.

Our results suggest that there are many advantages to being bold and proactive: proactive individuals have lower cortisol (chronically high cortisol can be harmful, Sapolsky et al. 1985, 1990) and they are better learners. Therefore what is maintaining timid individuals within this population? A popular idea from coping styles theory is that proactive individuals are better at learning novel contingencies, but become routinized and have a difficult time breaking away from established behavioral patterns when learning contingencies change (e.g., Bolhuis et al. 2004). According to this reasoning, reactive individuals are maintained within populations because they are behaviorally flexible, and perform better when the environment changes.

However, we found no evidence that proactive individuals were less behaviorally flexible: neither of our two measures of behavioral flexibility (number of mistakes and time to criterion during the reversal phase) were significantly correlated with the other traits. One

possible explanation for the failure to detect tradeoffs with behavioral flexibility is because we did not have sufficient statistical power to detect them. While the use of a high-resolution approach was very successful in producing a rich dataset that allowed us to simultaneously explore multiple facets of coping styles within each individual, it was extremely time intensive and resulted in a limited sample size. However, the observed correlation coefficients between behavioral flexibility and the other traits ranged from 0.4-0.46, or 'medium-large' effect sizes (Cohen 1992), and the direction of the relationships were *opposite* to those predicted.

A lack of positive results could also be due to the learning paradigm chosen. Reversal learning is commonly used to measure behavioral flexibility (Stamps 2015), but the exact behavioral mechanisms driving performance of this task are unclear (Izquierdo et al. 2012; 2016). Traditionally, performance during reversal learning was largely thought to reflect differences in inhibition control. However, more recent studies on rats of the underlying neural substrates suggest that differences related to sensory perception and awareness of unexpected results are likely to be involved (Chudasama et al. 2003; Schoenbaum et al. 2009). In other words, there might be multiple independent neural networks underlying reversal learning performance (Jentsch et al. 2014) and the facets linked to proactive/reactive behavioral types may be difficult to isolate with this paradigm. Additionally, tasks aimed at measuring attention sensitivity and behavioral inhibition separately (e.g., 5 choice serial reaction time task [5CSRTT]) in rats find that these behaviors can be manipulated independently (Chudasama et al. 2003; Paine et al. 2007). So perhaps the use of tasks aimed at isolating specific behavioral factors underlying reversal learning performance (e.g., 5CSRTT or barrier tasks) would provide better insights towards to coping styles predictions.

Another possibility for the failure to detect tradeoffs with behavioral flexibility is that it might not be as costly as is often assumed. While it has been suggested that there are cognitive trade-offs between novel learning and learning flexibility due to physiological and behavioral constraints (Chittka et al. 2009; Koolhaas et al. 1999; Sih and Del Giudice 2012), there are mixed results in the literature about the relationship between performance during initial and reversal learning (e.g., Bebus et al. 2016; Boogert et al. 2010; Guillette et al. 2015; Head et al. 1998) and some authors have suggested that flexibility and initial learning are independent (Ferrari et al. 2013; Koolhaas et al., 2010; Steimer et al. 1997), or even positively correlated (Ga1sworthy et al. 2002; Matzel et al. 2003). For example, as in humans (Plomin 2001), there might be a domain-general intelligence factor (*g*) in nonhuman animals that causes some individuals to be simply "smarter" relative to others in the population, and some individuals consistently do better on most cognitive tasks (Ga1sworthy et al. 2002; Matzel et al. 2003). Our findings are consistent with this idea, in that performance during initial and reversal learning were weakly (but nonsignificantly) *positively* correlated (r~0.4), and the individual that took the longest to reach criterion during the initial learning phase also took the longest to reach criterion during the reversal learning phase. Finally, it is possible that tradeoffs with behavioral flexibility do not help to maintain reactive individuals within populations, and there are other costs of being proactive such as increased vulnerability to predation (Bell and Sih 2007; Smith and Blumstein 2008).

There is growing interest in understanding the physiological and cognitive causes of behavioral variation. Testable theory such as coping styles provides a framework for understanding how and why different axes of variation are associated. Coping styles theory surmises that there is an entire suite of traits that distinguish between proactive and reactive

individuals. Until recently, coping styles research has largely focused on extreme, discrete variation that results from selective breeding programs. This study adds to the growing number of studies in a wide range of species that have developed paradigms (e.g., criterion-based learning: Guillette 2011; 2015; stress response: Boulton et al. 2015) that can accurately measure natural intraspecific variation of different facets of coping styles in a way that is objective and non-biased.

ACKNOWLEDGEMENTS

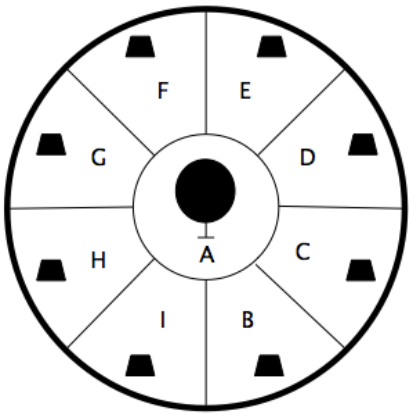
We are grateful to the members of the Bell lab for their help throughout this project, and we would like to specifically thank Wen Li Chen for her help collecting the water samples for the cortisol measures. We would also like to thank Sean Ehlman and Rebecca Halpin of the University of California Davis for their help with fish collection. Financial support was provided the National Science Foundation's Integrative Graduate Education and Research Traineeship program and by the University of Illinois.

COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflict of interest. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in this study involving animals were in accordance with the ethical standards of the University of Illinois, Urbana Champaign (IACUC protocol # 15077). This article does not contain any studies with human participants performed by any of the authors.

FIGURES

a)



b)

c)

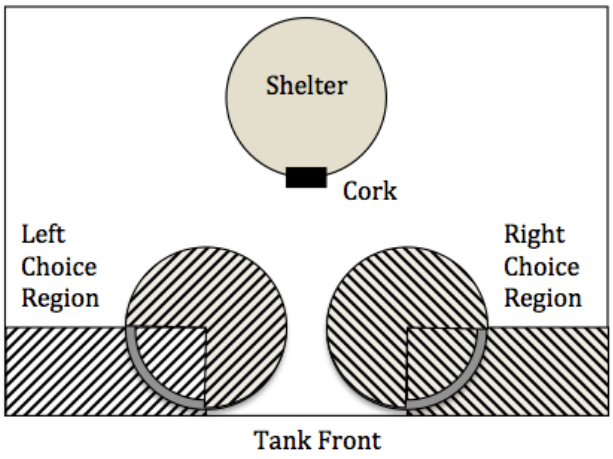
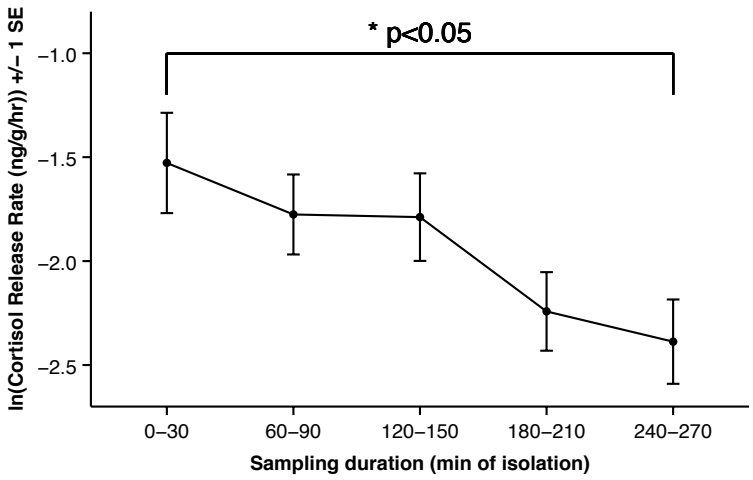


Figure 1.1. Experimental set up. a) Top-down view of tank set up for boldness assay. Kiddie pool was divided into 9 sections and marked A-I. Starting shelter was placed in the middle of section A. Black symbols in each section represent stones that were placed for investigation and additional shelter. **b) Photo of sculpin model used for boldness assay. c) Top-down view of the tank set up for training trials**. Fish were acclimated to environment by repeatedly being placed in the starting shelter and then allowed to make a choice. The same set up was also used for actual training trials. Striped regions show the choice regions. Once a fish entered either of these regions, its choice was noted.

a)



b)

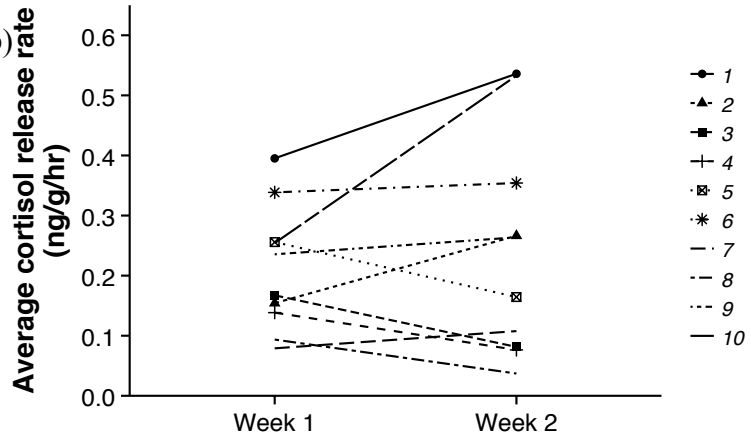
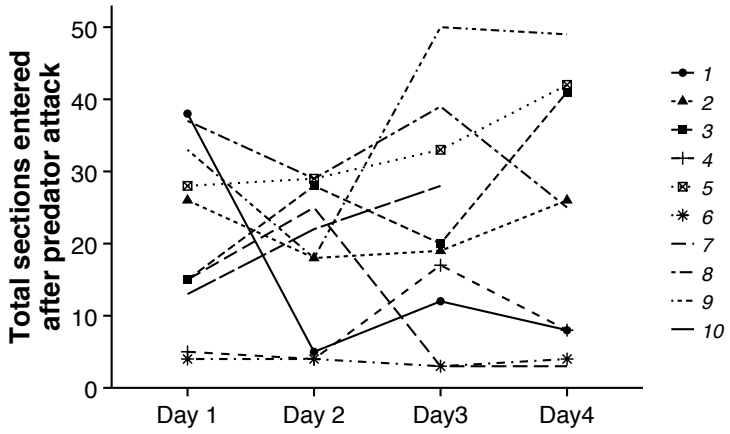


Figure 1.2. Cortisol repeated measures. a) Average cortisol release rate across the five sampling periods. Cortisol release rate was significantly lower 270 minutes after the start of isolation compared to 30 minutes (n=10). **b) Individual differences in average cortisol release rate across the two sampling weeks.** Each line represents an individual fish (n=10).

a)



b)

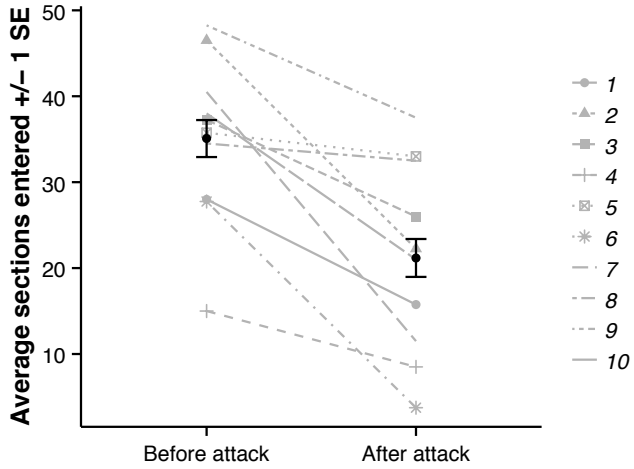


Figure 1.3. Boldness measures. a) Repeatability of boldness (total sections entered after predator attack) across four days. Each line represents an individual fish (n=10), with the same symbols for individuals as in Figure 1.2b. One individual (Fish 10) died after day 3. **b) Difference in activity before and after predator attack.** Fish moved less after the attack suggesting that predator model was perceived as a threat by the fish. The values on the y-axis represent the number of times each fish moved into a different section of the pool over the time observed. Grey lines indicate the average behavioral change in response to the predator attack for each individual fish, with the same symbols for individuals as in Figure 1.2b.

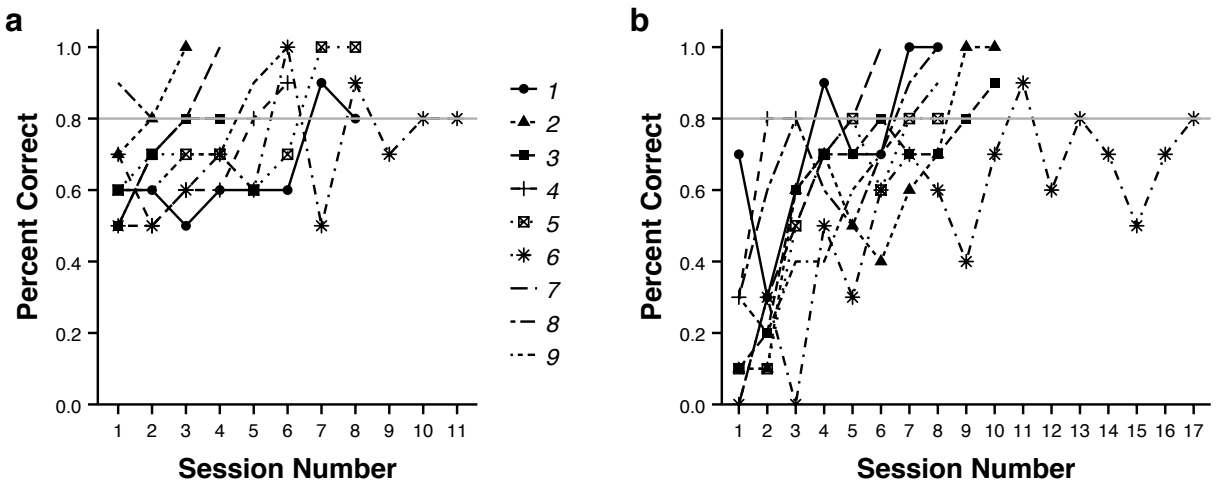


Figure 1.4. Individual learning curves during the a) initial learning, and b) reversal learning discrimination phases. Lines represent the performance of individuals across sessions, with the same symbols for individuals as in Figure 1.2b. Criterion was reached when the proportion of correct choices was greater than or equal to 0.8 (marked by horizontal grey line) on two consecutive sessions. Note the poor performance during the first and second sessions of the reversal learning phase, suggesting that fish initially relied on the previously-learned association.

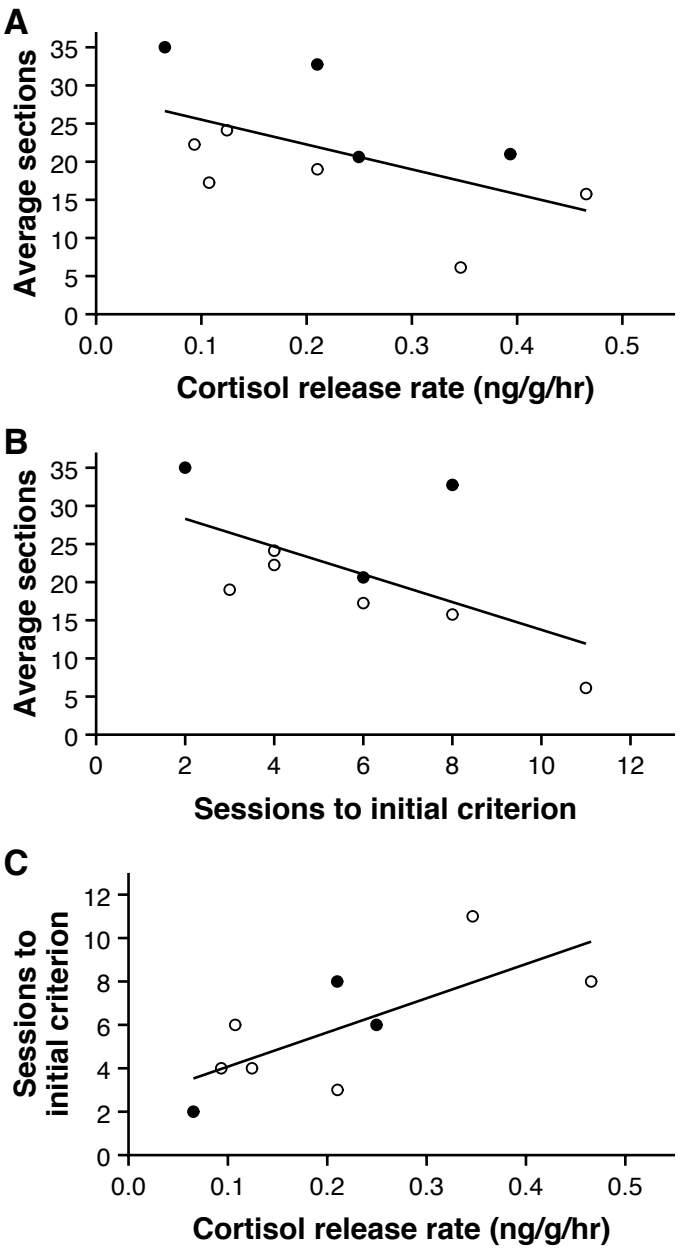


Figure 1.5. Scatterplots showing results consistent with coping styles theory. Each data point represents a different individual, coded by sex (open circles = females, closed circles = males) a) Bolder individuals (i.e., fish that entered more sections after the predator attack) had lower average cortisol release rates across two weeks. b) Bolder individuals reached criterion in the initial color discrimination task in fewer sessions. c) Individual fish with higher average cortisol release rates took more sessions to reach criterion in the initial color discrimination task.

REFERENCES

Agrillo C, Petrazzini MEM, Piffer L, Dadda M, Bisazza A (2012). A new training procedure for studying discrimination learning in fish. Behav Brain Res 230:434-343-348

Aubin-Horth N, Deschênes M, Cloutier S (2012) Natural variation in the molecular stress network correlates with a behavioural syndrome. Horm Behav 61:140-146

Barreto RE Volpato GL (2011) Ventilation rates indicate stress-coping styles in Nile tilapia. J Biosci 36:851-855

Bebus SE, Small TW, Jones BC, Elderbrock EK, Schoech SJ (2016) Associative learning is inversely related to reversal learning and varies with nestling corticosterone exposure. Anim Behav 111:251-260

Bell AM (2005) Behavioural differences between individuals and two populations of stickleback (Gasterosteus aculeatus). J Evol Biol 18:464-73

Bell AM, Hankison SJ, Laskowski KL (2009) The repeatability of behaviour: a meta-analysis. Anim Behav 77:771-83

Bensky MK, Gosling SD, Sinn DL (2013) The world from a dog's point of view: a review and synthesis of dog cognition research. Adv Study Anim Behav 45:209-406

Benus RF, Den Daas S, Koolhaas JM, Van Oortmerssen GA (1990) Routine formation and flexibility in social and non-social behaviour of aggressive and non-aggressive male mice Behaviour 112:176-193

Bolhuis JE, Schouten WG, de Leeuw JA, Schrama JW, Wiegant VM (2004) Individual coping characteristics, rearing conditions and behavioural flexibility in pigs. Behav Brain Res 152:351-360

Bond AB, Kamil AC, Balda RP (2007) Serial reversal learning and the evolution of behavioral flexibility in three species of North American corvids (*Gymnorhinus cyanocephalus, Nucifraga columbiana, Aphelocoma californica*). J Comp Psychol 121:372-379

Boogert NJ, Reader SM, Laland KN (2006) The relation between social rank, neophobia and individual learning in starlings. Anim Behav 72:1229-1239

Boulton K, Couto E, Grimmer AJ, Earley RL, Canario AVM, Wilson AJ, Walling CA (2015) How integrated are behavioral and endocrine stress response traits? A repeated measures approach to testing the stress-coping style model. Ecol Evol 5:618-633

Brown C, Braithwaite VA (2004) Size matters: a test of boldness in eight populations of the poeciliid Brachyraphis episcopi. Anim Behav 2004 68:1325-9

Brown C, Burgess F, Braithwaite VA (2007) Heritable and experiential effects on boldness in a tropical poeciliid. Behav Ecol Sociobiol 62:237-43

Budaev S, Brown C (2011) Personality traits and behaviour. In Brown CKL, Krause J (Eds.), Fish cognition and behaviour (2nd ed., pp. 135–156). Cambridge, United Kingdom: Blackwell

Canestrelli D, Bisconti R, Carere C (2016) Bolder takes all? The behavioral dimension of biogeography. Trends Ecology Evolut 31: 35-43

Carazo P, Noble DWA, Chandrasoma D, Whiting MJ (2014) Sex and boldness explain individual differences in spatial learning in a lizard. Proc R Soc B 281:20133275

Carere C, Caramaschi D, Fawcett TW (2010) Covariation between personalities and individual differences in coping with stress: converging evidence and hypotheses. Curr Zool 56: 728-740

Carere C, Locurto C (2011) Interaction between animal personality and animal cognition. Curr Zool 57:491-498

Chittka L, Skorupski P, Raine NE (2009) Speed accuracy trade-offs in animal decision making. Trends Ecology Evolut 24:400-407

Chudasama Y, Robbins TW (2003) Dissociable contributions of the orbitofrontal and infralimbic cortex to pavlovian autoshaping and discrimination reversal learning: further evidence for the functional heterogeneity of the rodent frontal cortex. J Neurosci 23: 8771-8780

Cockrem JF, Barrett DP, Candy EJ, Potter MA (2009) Corticosterone responses in birds: individual variation and repeatability in Adelie penguins (Pygoscelisadeliae) and other species, and the use of power analysis to determine sample sizes. Gen Comp Endocrin 163:158-68

Cohen J (1992) A power primer. Psychol Bull 112:155-159

Cole EF, Quinn JL (2011) Personality and problem-solving performance explain competitive ability in the wild. Proc R Soc B, rspb20111539

Costantini D, Ferrari C, Pasquaretta C, Cavallone E, Carere C, von Hardenberg A, Réale D (2012) Interplay between plasma oxidative status, cortisol and coping styles in wild alpine marmots, Marmota marmota. J Exp Biol 215: 374-383

Cook KV, O'Connor CM, McConnachie SH, Gilmour KM, Cooke SJ (2012). Condition dependent intra-individual repeatability of stress-induced cortisol in a freshwater fish. Comp Biochem Phys A 161:337-43

de Boer SF, Buwalda B, Koolhaas JM (2016) Untangling the neurobiology of coping styles in rodents: Towards neural mechanisms underlying individual differences in disease susceptibility. Neurosci Biobehav Rev

Dingemanse NJ, Wright J, Kazem AJ, Thomas DK, Hickling R, Dawnay N (2007) Behavioural syndromes differ predictably between 12 populations of three-spined stickleback. J Anim Ecol 76:1128-38

Dugatkin LA, Alfieri MS (2003) Boldness, behavioral inhibition and learning. Ethol Ecol Evol 15:43-39

Ferrari C, Pasquaretta C, Carere C, Cavallone E, von Hardenberg A, & Réale D (2013) Testing for the presence of coping styles in a wild mammal. Anim Behav 85:1385-1396

Frost AJ, Winrow-Giffen A, Ashley PJ, Sneddon LU (2007) Plasticity in animal personality traits: does prior experience alter the degree of boldness? Proc R Soc B 274: 333-339

Fürtbauer I, Pond A, Heistermann M, King AJ (2015) Personality, plasticity and predation: linking endocrine and behavioural reaction norms in stickleback fish. Funct Ecol 29: 931-940

Galsworthy MJ, Paya-Cano JL, Monleón S, Plomin R (2002) Evidence for general cognitive ability (g) in heterogeneous stock mice and an analysis of potential confounds. Genes Brain Behav 1:88-95

Girvan JR, Braithwaite VA (1998) Population differences in spatial learning in three–spined sticklebacks. Proc R Soc B 265:913-918

Griffen AS, Guillette LM, Healy SD (2015) Cognition and personality: an analysis of an emerging field. Trends Ecol Evol 30:207-214

Guillette LM, Hahn AH, Hoeschele M, Przyslupski AM, Sturdy CB (2015) Individual differences in learning speed, performance accuracy and exploratory behaviour in black-capped chickadees. Anim Cogn 18:165-178

Guillette LM, Reddon AR, Hoeschele M, Sturdy CB (2011) Sometimes slower is better: slow-exploring birds are more sensitive to changes in a vocal discrimination task. Proc R Soc B 278:767-773

Hadfield JD (2010) MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R package. J Stat Softw 33:1-22

Harris S, Ramnarine IW, Smith HG, Pettersson LB (2010) Picking personalities apart: estimating the influence of predation, sex and body size on boldness in the guppy Poecilia reticulata. Oikos. 119:1711-8

Hennessy MB. Voith VL, Mazzei SJ, Buttram J, Miller DD, Linden F (2001) Behavior and cortisol levels of dogs in a public animal shelter, and an exploration of the ability of these measures to predict problem behavior after adoption. Appl Anim Behav Sci 73:217-233

Herrmann E, Hernandez-Lloreda MV, Call J, Hare B, Tomasello M (2009) The structure of individual differences in the cognitive abilities of children and chimpanzees. Psychol Sci 21:102-110

Huntingford FA (1976) The relationship between anti-predator behaviour and aggression among conspecifics in the three-spined stickleback, *Gasterosteus aculeatus*. Anim Behav 24:245-60

Izquierdo A, Brigman JL, Radke AK, Rudebeck PH, Holmes A (2016) The neural basis of reversal learning: An updated perspective. Neurosci

Izquierdo A, Jentsch JD (2012) Reversal learning as a measure of impulsive and compulsive behavior in addictions. Psychopharmacology 219: 607-620

Jentsch JD, Ashenhurst JR, Cervantes MC, Groman SM, James AS, Pennington ZT (2014) Dissecting impulsivity and its relationships to drug addictions. Ann N Y Acad Sci 1327: 1-26

King AJ, Fürtbauer I, Mamuneas D, James C, Manica A (2013) Sex-differences and temporal consistency in stickleback fish boldness. PloS one 8: e81116

Kosten TA, Kim JJ, Lee HJ (2012) Early life manipulations alter learning and memory in rats. Neuro Biobehav Rev 36:1985-2006

Koolhaas JM, De Boer SF, Coppens CM, Buwalda B (2010) Neuroendocrinology of coping styles: towards understanding the biology of individual variation. Front Neuroendocrin 31: 307-321

Koolhaas JM, Korte SM, De Boer SF, Van Der Vegt BJ, Van Reenen CG, Hopster H, De Jong IC, Ruis MAW, Blokhuis HJ (1999) Coping styles in animals: current status in behavior and stress – physiology. Neurosci Biobehav Rev 23:925-936

Locurto C (2007) Individual differences and animal personalities. Comp Cogn Behav Rev 2:67-78

Lupien SJ, McEwen BS (1997) The acute effects of corticosteroids on cognition: integration of animal and human model studies. Brain Res Rev 24:1-27

Lupien SJ, de Leon M, De Santi S, Convit A, Tarshish C, Nair NPV, Thakur M, McEwen BS, Hauger RL, Meaney MJ (1998) Cortisol levels during human aging predict hippocampal atrophy and memory deficits. Nat Neurosci 1: 69-73

Lupien SJ, McEwen BS, Gunnar MR, Heim C (2009) Effects of stress throughout the lifespan on the brain, behaviour and cognition. Nat Rev Neuro 10:434-445

Mamuneas D, Spence AJ, Manica A, King AJ (2015) Bolder stickleback fish make faster decisions, but they are not less accurate. Behav Ecol 26:91-96

Matzel LD, Han YR, Grossman H, Karnik MS, Patel D, Scott N, et al. (2003) Individual differences in the expression of a "general" learning ability in mice. J Neurosci 23:6423-6433

McEwen BS, Sapolsky RM (1995) Stress and cognitive function. Curr Opin Neurobiol 5:205-16.

Mesquita FO, Borcato FL, Huntingford FA (2015) Cue-based and algorithmic learning in common carp: a possible link to stress coping style. Behav Process 115:25-29

Niemela PT, Vainikka A, Forsman JT, Loukola OJ, Kortet R (2013) How does variation in the environment and individual cognition explain the existence of consistent behavioral differences. Ecol Evol 2:457-464

Nippak PMD, Milgram NW (2005) An investigation of the relationship between response latency across several cognitive tasks in the beagle dog. Prog Neuropsychopharmacol Biol Psychiatry 29:371-377

Odling-Smee L, Braithwaite VA (2003) The influence of habitat stability on landmark use during spatial learning in the threespined stickleback. Anim Behav 65:701-707

Ouyang JQ, Hau M, Bonier F (2011) Within seasons and among years: when are corticosterone levels repeatable? Horm Behav 60:559-64

Øverli Ø, Pottinger TG, Carrick TR, Øverli E, Winberg S (2002) Differences in behaviour between rainbow trout selected for high-and low-stress responsiveness. J Exp Biol 205:391-5

Øverli Ø, Sørensen C, Pulman KG, Pottinger TG, Korzan W, Summers CH, Nilsson GE (2007) Evolutionary background for stress-coping styles: relationships between physiological, behavioral, and cognitive traits in non-mammalian vertebrates. Neurosci Biobehav Rev 31:396-412

Øverli Ø, Winberg S, Pottinger TG (2005) Behavioral and neuroendocrine correlates of selection for stress responsiveness in rainbow trout—a review. Integ Comp Biol 45:463-74

Paine TA, Tomasiewicz HC, Zhang K, Carlezon WA (2007). Sensitivity of the five-choice serial reaction time task to the effects of various psychotropic drugs in Sprague-Dawley rats. Biol Psychiatry 62: 687-693

Pintor L, McGhee KE, Roche DP, Bell AM (2014) Individual variation in foraging behavior reveals a tradeoff between flexibility and performance in a top predator. Behav Ecol Sociobiol 68:1711-1722

Plomin R (2001) The genetics of g in human and mouse. Nat Rev Neurosci 2:136-141

Pottinger TG, Carrick TR (1999) Modification of the plasma cortisol response to stress in rainbow trout by selective breeding. Gen Comparative Endocrin 116:122-32

Pressley PH (1981) Parental effort and the evolution of nest-guarding tactics in the threespine stickleback, Gasterosteus aculeatus L. Evolution 35:282-295

Range F, Hentrup M, Viranyi Z (2011) Dogs are able to solve a means-end task. Anim Cogn 14:575-583

Raoult V, Brown C, Zuberi A, Williamson JE (2012). Blood cortisol concentrations predict boldness in juvenile mulloway (Argyosomus japonicus). J Ethol 30:225-232

Roberts ML, Buchanan KL, Hasselquist D, Bennett AT, Evans MR (2007) Physiological, morphological and behavioural effects of selecting zebra finches for divergent levels of corticosterone. J Exp Biol 210:4368-78

Ruiz-Gomez ML, Huntingford FA, Øverli Ø, Thornqvist P-O, Hoglund E (2011) Response to environmental change in rainbow trout selected for divergent stress coping styles. Physiol Behav 102:317-322

Sapolsky RM, Krey LC, McEwen BS (1985) Prolonged glucocorticoid exposure reduces hippocampal neuron number: implications for aging. J Neurosci 5:1222–1227

Sapolsky RM, Uno H, Rebert CS, Finch CE (1990) Hippocampal dam- age associated with prolonged glucocorticoid exposure in primates. J Neurosci 10:2897–2902

Sapolsky RM, Romero LM, Munck AU (2000) How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions 1. Endocr Rev 21:55-89

Schoech SJ, Rensel MA, Heiss RS (2011) Short-and long-term effects of developmental corticosterone exposure on avian physiology, behavioral phenotype, cognition, and fitness: a review. Curr Zool 57:514-30

Schoenbaum G, Roesch MR, Stalnaker TA, Takahashi YK (2009) A new perspective on the role of the orbitofrontal cortex in adaptive behaviour. Nat Rev Neurosci 10: 885-892

Sebire M, Katsiadaki I, Scott AP (2007) Non-invasive measurement of 11-ketotestosterone, cortisol and androstenedione in male three-spined stickleback (Gasterosteus aculeatus). Gen Comp Endocr 152:30-38

Sebire M, Katsiadaki I, Scott AP (2009) Further refinement of the non-invasive procedure for measuring steroid production in the male three-spined stickleback *Gasterosteus aculeatus*. J Fish Biol 75:2082-2094

Shettleworth SJ (2010) Cognition, evolution, and behavior (2nd Ed.) Oxford University Press, New York

Sih A, Del Giudice M (2012) Linking behavioural syndromes and cognition: a behavioural ecology perspective. Philos Trans R Soc B 367:2762-2772

Smith BR, Blumstein DT (2008) Fitness consequences of personality: a meta-analysis. Behav Ecol 19:448-55

Sneddon LU (2003) The bold and the shy: individual differences in rainbow trout. J Fish Biol 62: 971-975

Sorensen C, Johansen IB, Øverli Ø (2013) Neural plasticity and stress coping in teleost fishes. Gen Comp Endocrinol 181:25-34

Stamps JA (2015) Individual differences in behavioural plasticities. Biol Rev 91:534-567

Stein LR, Bell AM (2012) Consistent individual differences in fathering in threespined stickleback *Gasterosteus aculeatus*. Curr Zool 58:45-52

Thomson JS, Watts PC, Pottinger TG, Sneddon LU (2012) Plasticity of boldness in rainbow trout, Oncorhynchus mykiss: do hunger and predation influence risk-taking behaviour? Horm Behav 61: 750-757

Thornton A, Lukas D (2012) Individual variation in cognitive performance: developmental and evolutionary perspectives. Philos Trans R Soc B 367:2773-2783

Tudorache C, Schaaf MJ, Slabbekoorn H (2013) Covariation between behaviour and physiology indicators of coping style in zebrafish (Danio rerio). J Endocrin 219:251-258

Verbeek ME, Drent PJ, Wiepkema PR (1994) Consistent individual differences in early exploratory behaviour of male great tits. Anim Behav 48:1113-1121

Wolf M, Sander van Doorn G, Weissing FJ (2008) Evolutionary emergence of responsive and unresponsive personalities. Proc. Natl Acad. Sci. 105: 15825-15830

CHAPTER 2: INTRASPECIFIC VARIATION IN CUE-SPECIFIC LEARNING IN STICKLEBACKS[2]

ABSTRACT

Animals must identify reliable cues amidst environmental noise during learning, and the cues that are most reliable often depend on the local ecology. Comparing the performance of populations of the same species across multiple versions of a cognitive task can reveal whether some populations learn to use certain cues faster than others. Here, using a criterion-based protocol, we assessed whether two natural populations of sticklebacks differed in how quickly they learned to associate two different discrimination cues with the location of food. One version of the discrimination task required animals to use visual (colour) cues while the other required animals to use egocentric (side) cues. There were significant behavioural differences between the two populations, but no evidence that one population was generally better at learning, or that one version of the task was generally harder than the other. However, the two populations excelled on different tasks: fish from one population performed significantly better on the side version than they did on the colour version, while the opposite was observed in the other population. These results suggest that the two populations are equally capable of discrimination learning, but are primed to form associations with different cues. Ecological differences between the populations in environmental stability might account for the observed variation in learning.

[2] Chapter 2 was published in its entirety by Animal Behaviour. Bensky, M. K., Bell, A. M. (2018). Intraspecific variation in cue-specific learning in sticklebacks. Animal Behaviour 137: 161-168. This article is reprinted with permission of the publisher and is available from https://doi.org/10.1016/j.anbehav.2018.01.003

These findings highlight the value of comparing cognitive performance on different variations of the same task in order to understand variation in cognitive mechanisms.

INTRODUCTION

There are multiple biotic and abiotic stimuli in the environment that might be associated with factors important for fitness such as food, predators, or conspecifics. When attentional capacities are limited, animals must decide which cues to prioritize, and this can lead to selective attention toward certain cues over others (Gottselig et al. 2001). Animals often learn to associate salient cues with rewarding (or adverse) behavioural outcomes faster than they do with non-salient cues (Mackintosh 1975; Rescorla and Wagner 1972; Treviño 2016). Correctly identifying cues that are the most reliable and relevant to specific outcomes can improve fitness. Provided that there is genetic variation for sensitivity to environmental cues (e.g., Visser et al. 2011), the cognitive mechanisms that prime organisms to attend to the most ecologically-relevant stimuli are likely to be honed by natural selection, thereby leading to adaptive differences in the rate at which animals that inhabit different environments learn to associate different types of cues with behavioural outcomes.

Indeed, animals are often primed to learn to associate certain environmental cues with specific outcomes faster than others. For example, young birds are predisposed to learn species-specific over heterospecific songs (reviewed in Wheatcroft and Qvarnström 2015). Similarly, dogs prioritize attending to egocentric cues (i.e., cues relative to their body position) over allocentric cues (i.e., landmarks) to locate objects (Fiset et al. 2000). Selection should favor cue-specific learning when certain combinations of cues and outcomes are more likely to occur in

nature. For example, rats quickly learned to associate an olfactory cue (but not an auditory cue) with sickness, possibly because reliable associations between olfactory cues and food-related illness are more likely to occur in nature (i.e., selective association; Garcia and Koelling 1966). These examples suggest that differential associative learning speeds between different types of cues are likely to be adaptive and widespread. Adaptive, fine scale variation in cue-specific learning has implications for the evolution of cognition. One promising tactic for investigating this is to compare the rates at which populations of the same species from different environments learn to associate different cues with a reward.

Threespined stickleback (*Gasterosteus aculeatus*) are good subjects for studying the adaptive significance of intraspecific variation in cognition. Sticklebacks occupy a variety of different freshwater environments that vary in water clarity, predation intensity, habitat complexity and stability. Sticklebacks exhibit tremendous intraspecific diversity in morphological, physiological, behavioural and cognitive traits (Bell and Foster 1994). Indeed, a number of studies have reported ecologically-driven variation in cue use during learning in sticklebacks (Braithwaite and Girvan 2003; Brydges et al. 2008; Duffy et al. 2009; Girvan and Braithwaite 1998; Odling-Smee and Braithwaite 2003; Odling-Smee et al. 2008) and have suggested that populations have biases for specific spatial and/or environmental (e.g., water flow) cues. For example, sticklebacks from rivers (where visual landmarks are likely to change quickly), tended to rely more on movement-based algorithms (e.g., always turn left or use a specific turn sequence) to locate food, while sticklebacks from ponds (where visual landmarks are more likely to be stable and reliable) depended more on visual landmarks to complete the maze (Girvan and Braithwaite 1998; Odling-Smee et al. 2003).

While these findings are consistent with the hypothesis that there is adaptive intraspecific

variation in cue use during learning, the experiments did not specifically isolate the cues available during learning, and therefore the studies were unable to decisively conclude that populations learned to follow certain cues faster than others. For example, Odling-Smee and Braithwaite (2003) trained sticklebacks from ponds and streams in a T-maze to follow a combination of landmark and egocentric cues (e.g., food was always at the end of the left arm of the maze and a plant was always placed towards the left arm). Once a fish reached criterion, a probe trial put the two types of cues into conflict (e.g., food was moved to the right arm, but the plant landmark remained near the left arm). Consistent with the hypothesis of ecologically-driven differences in cue use, the pond and river populations differed in the proportion of individuals that used landmark versus egocentric cues. However, because the two types of cues were conflated during the learning trials that preceded the probe trial, whether the populations differed in how quickly they associated one versus the other type of cue with the reward is unknown. Similarly, Girvan and Braithwaite (1998) trained sticklebacks from different populations to navigate a maze either with or without visual plant landmarks. In both treatments, the turn-sequence for solving the maze was the same across trials, which meant that the egocentric and allocentric cues were redundant in the landmark-trained group. Therefore, differences in learning speeds could not be attributed to a specific cue (for an example with isolated spatial cues that measures learning speeds in goldfish see Rodriguez et al 1994).

Here we build upon these findings by comparing the learning performance of sticklebacks from two populations on two versions of the same task that differ in the particular discriminatory cue that is available to use during learning. In one version, sticklebacks could use visual (colour) cues to solve the task. In the other version, sticklebacks could use egocentric spatial (side) cues to solve the task. Spatial memory and colour discrimination likely involve

dissociated regions of the brain (Hampton and Shettleworth 1996), and brain regions associated with spatial memory are evolutionarily conserved in vertebrates (Rodriguez et al. 2002). Thus, comparing performance between the colour and side versions of the task may provide insights into how ecological variation can drive variation in learning that involve partially independent brain regions. We used a criterion-based protocol which allowed us to quantify how quickly individuals learned to follow a particular discriminatory cue. While previous studies suggested that populations have preferences for different cues (Girvan and Braithwaite 1998; Odling-Smee et al. 2003), our experiment explicitly tests whether populations differ in how quickly they associate different discriminatory cues with reward outcomes. We assume that individuals learn to form associations more readily with cues that are more reliable in their environment.

We measured fish from two populations from two different drainages in Northern California: Putah Creek and Navarro River. There are phenotypic differences between fish from these populations (Bell 2005) and the sites are different ecologically. For example, there is more vegetation and less water movement at the Putah site, while the Navarro River is much clearer, with more dramatic seasonal changes in water flow. We hypothesize that ecological differences between the two populations cause differences in the predictability and reliability of different cues, thereby causing the populations to have different learning speeds in cue-specific learning. In particular, based on the work by Girvan and Braithwaite (1998), and Odling-Smee and Braithwaite (2003), we predicted that fish from the Navarro River, where visual cues are unlikely to persist over time due to changes in water flow, would excel when given an opportunity to use egocentric spatial cues and that the fish from Putah Creek, where there is less water flow, would perform well when given an opportunity to use visual cues. To address the possibility that population differences in cognitive performance reflect differences among individuals and/or

between populations in acclimation to the lab and/or boldness, we also recorded acclimation time and latency to emerge from a shelter during training.

METHODS

Wild-caught, non-gravid adult female threespined sticklebacks from two separate drainages (Putah Creek, CA, USA and Navarro River, CA, USA) were randomly assigned to either a colour or side version of a discrimination learning task. On average, sticklebacks from Putah Creek are larger than sticklebacks from the Navarro River (Length - Putah: 45.5 ± 0.803 mm; Navarro: 40.83 ± 0.690 mm; $t = -4.407$, $df = 58$, $p < 0.0001$). Sticklebacks were housed individually in 26.5 L tanks (36 cm long x 33 cm wide x 24 cm high).

Pretraining

To ensure similar levels of motivation and reduce potential sampling bias, all individuals were run through a two-phase criterion-based pre-training protocol. The first pretraining phase involved training the sticklebacks to search cups for food. Two translucent cups were placed in an individual's home tank and half the daily ration of bloodworms (5 worms per cup) were placed in petri dishes at the bottom of each cup. A quarter of the cups' sides were cut out to provide access to the petri dishes. This step was repeated once a day until the individual ate from both cups within ten minutes on three consecutive days.

The second pre-training phase involved acclimating the sticklebacks to the process of being repeatedly placed into a starting shelter from which they had to emerge to search the cups for food. Habituating them to the shelter was important because it allowed us to standardize

individuals' starting location across trials. During each trial, an individual was gently transported into a shelter in a separate 26.5 L training tank (36 cm long x 33 cm wide x 24 cm high) and left to rest for one minute. Translucent cups were placed in the tank. Olfactory cues of food were present in both cups, but food was only accessible in one cup. Specifically, there were bloodworms in a petri dish in the bottom of each cup, but one of the petri dishes had strips of electrical tape covering the entire opening which prevented the fish from accessing the worms. Several small holes were punctured into the tape to allow chemical/odor cues to emanate from the dish. The other (rewarded) dish had the same amount of electrical tape lining the outside edge, but access to the contents was not obstructed. Each fish received two trials per day, with the location of the rewarded cup alternating between the two trials. To start each trial, a cork was removed from the shelter to allow the stickleback to exit. We measured how long it took the individual to emerge from the shelter (*time to emerge)*. We interpret *time to emerge* as willingness to take risks ("boldness"; Wilson and Godin 2009). The second pretraining phase continued until the individual found and ate the food in under five minutes during each trial on three consecutive days. We refer to the total number of days to complete both phases of pretraining as *days to complete pretraining.* The minimum number of days to pass pretraining was six days. All fish passed the pre-training phase. We infer that if a fish was willing to search and consume a food reward relatively quickly during pretraining that it was well-acclimated to the learning environment. We tested whether differences in 'boldness' (*time to emerge*) and acclimation to the testing environment could explain differences in learning performance among individuals and populations.

Training

Training took place in the same 26.5 L training tank used for the second phase of pre-training. Individuals from both populations were randomly assigned to one of two training conditions: 1) blue-yellow colour discrimination, or 2) left-right side discrimination. Sticklebacks received one 10-trial learning session per day and were trained in only one of the two conditions.

Colour discrimination

In this version of the discrimination task, sticklebacks were presented with a yellow and blue cup and were trained to associate the blue cup with a food reward. Stickleback are known to discriminate between these two colours (Bensky et al. 2017, Feng et al. 2015; Roche et al. 2012), and pilot experiments found no evidence of a colour bias in either of the two populations (Average proportion of preference trials chose blue - Navarro: mean = 0.39, sd = 0.303, t_9 = 1.1595, p = 0.2761; Putah: mean = 0.44, sd = 0.227, t_9 = 0.8358, p = 0.4249). The stickleback was placed in the shelter at the back of the tank. While in the shelter, a blue and yellow cup were placed at the front of the tank (Figure B.1a). A petri dish was then placed into the base of each cup. The blue cup was rewarded with 1-2 bloodworms. To control for the possible use of olfactory/chemical discriminatory cues, as described *Pretraining*, the dish in the unrewarded yellow cup also contained 1-2 bloodworms and was covered with electrical tape to prevent access to the worms, while the dish in the blue cup had tape wrapped around the outside edge. Across trials, the location of the blue cup was pseudo-randomized for each individual fish according to the following two rules: 1) each side was rewarded in half of the trials, and 2) a single side could not be rewarded more than two trials in a row. After the cups were in place, the

cork of the shelter was removed. For each trial four variables were recorded: 1) time to emerge from the shelter, 2) time to enter the choice region of the first cup searched (see Figure B.1a), 3) time to enter the choice region of the correct cup, and 4) if the first choice was the correct choice. Regardless of whether the first choice was correct, the fish was allowed to explore the tank until it searched the blue cup and was given a chance to eat the bloodworms. Therefore, all individuals were given an equal opportunity to associate the rewarded stimulus cue with the food reward, regardless of their first choice and how quickly they explored the cups. The colour training protocol continued until the fish met criterion (two consecutive sessions of at least 8 correct choices out of 10 trials), hereafter referred to as *sessions to criterion*.

Side discrimination

In this version of the discrimination task, sticklebacks were presented with two identical cups (blue) and trained to associate a particular direction (left or right) with a food reward. This task took place in a tank identical to the tank used for colour discrimination. Here, though, across trials, the shelter was moved from one side of the tank to the other with the opening facing the opposite side (Figure B.1b). The shelter's location for any given trial was determined by the same pseudo-randomization rules used for the colour discrimination training. The rewarded cup was based on its orientation relative to the opening of the shelter. Fish were either trained to always choose the cup that was always to the left of the opening or were trained to always choose the cup on the right. In order to increase the difficulty of the task, individuals were always trained to the side they selected less often during pre-training. If there was no preference during pre-training, the fish were randomly assigned to a side. For both populations, 8 fish were trained to the left and 7 fish were trained to the right. The side discrimination training protocol

continued until the fish met criterion (two consecutive sessions of at least 8 correct choices out of 10 trials).

62 females started the training phase. Of those, 60 fish (30 per population) met criterion (N = 2 Navarro fish were removed due to reduced food motivation during training), with the resulting sample size comprising n = 15 in each of the four combinations of tasks and populations.

Statistical analysis

Due to size differences between populations, and the possible links between size and within-population behavioural variation (Adriaenssens and Johnsson 2011; Gill and Hart 1994), all analysis initially included fish length as a covariate. However, length was never significant (P>0.05) and was therefore omitted from subsequent analyses.

Days to complete pretraining was non-normal and normality was not improved by transformations, therefore population differences in *days to complete pretraining* were compared using a nonparametric permutation test simulating 100,000 samples. We used a t-test (R Core Team 2016; package = "stats"; function = "t.test") to compare the two populations' average *time to emerge* across the first ten training trials. We focus on these early trials because both populations habituated to the training protocol and emerged quickly during later trials. To examine whether fish from the two different populations differed in their performance on the colour and side versions of the discrimination task we took two approaches. First, to determine if the populations differed in the rate at which individuals met the learning criterion, we conducted a survival analysis using a Cox proportional hazards regression model (Therneau 2015; package = "survival"; function = "coxph") where population, version (colour vs side), and their

interaction were included as fixed effects. Failure to reach criterion during a session was treated as "survival". Second, we compared how quickly individuals reached criterion on their designated training task using a two-way between-groups analysis of variance (ANOVA) with population, version, and their interaction as fixed effects (R Core Team 2016; package = "stats"; functions = "lm"; "anova"). T-tests were used to examine *a priori* contrasts between and within populations across both tasks.

To test if individual differences in boldness (*time to emerge*) were related to learning performance, we used multiple regression with population, version, and average time to emerge across the first 10 trials as predictors of time to criterion ((R Core Team 2016; package = "stats"; function = "lm"). *Time to emerge* and *sessions to criterion* were log-transformed to meet assumptions of normality. All statistical analyses were carried out using R 3.1 (R Core Team 2016). Figures were made utilizing the graphics (R Core Team 2016) and ggplot2 packages (Wickham 2009).

Ethical Note

The experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Illinois Urbana-Champaign. Fish were caught in the field using baited minnow traps under the approval of the State of California's Department of Fish and Wildlife permit to A.B. Bell (#SC-3310). In the lab, the fish were housed in groups before and after the experiment. While the fish were undergoing training they were housed individually, but were given visual access to neighboring fish when they were not participating in active trials in order to enhance their welfare. All experimental procedures were non-invasive.

RESULTS

Population differences in behaviour

On average, sticklebacks from Putah Creek acclimated faster to the lab and emerged faster from a shelter than sticklebacks from the Navarro River. For example, sticklebacks from Putah Creek completed pretraining faster (median = 7 days, IQR = 2, N = 30), than sticklebacks from the Navarro River (median = 11 days, IQR = 5.75, N = 30), and the difference between the medians was highly significant (permutation test: 1/100,000 samples ≥ observed difference = 4 days, $p<0.001$; Figure 2.1). Similarly, sticklebacks from Putah Creek emerged significantly faster from the starting shelter during the first ten training trials compared to sticklebacks from the Navarro River (Putah: mean = 15.62 s, sd = 11.27; Navarro: mean = 31.98 s, sd = 27.95; t_{58} = 2.98, p = 0.004; Figure 2.2).

Population differences in learning performance

Learning performance (*sessions to criterion*) depended on both population and version of the discrimination task. Specifically, there was a statistically significant interaction between population and version on learning performance according to both the survival analysis (β = -1.6124, z = -2.878, p = 0.004, Figure 2.3a, b) and ANOVA ($F_{1,56}$ = 12.45, $p < 0.001$, Figure 2.3c) models. Populations performed better on one of the two versions of the task, but the populations differed in the version on which they excelled. Sticklebacks from Putah Creek performed better on the colour discrimination version than on the side discrimination version (t_{28} = 2.62, p = 0.014; colour discrimination: mean = 3.87 days, sd = 1.30; side discrimination: mean = 6.47 days, sd = 3.76). Sticklebacks from the Navarro River, on the other hand, performed better on the

side discrimination version than the colour discrimination version ($t_{28} = 2.41$, $p = 0.023$; colour discrimination: mean = 7.53 days, sd = 3.40; side discrimination: mean = 5.0, sd = 3.14). There was no indication that sticklebacks from one population had overall better learning performance than sticklebacks from the other population, or that one version of the task was more difficult than the other: when the population by version interaction term was removed from the model, there was not a main effect of either population ($F_{1,57} = 1.29$, $p = 0.26$) or version ($F_{1,57} = 0.039$, $p = 0.85$) on learning performance.

DISCUSSION

If animals prioritize attention to the most ecologically relevant cues, then animals inhabiting different environments might rely on different cues during learning. Here, we measured the learning performance of sticklebacks from two populations on different versions of a discrimination task. One version required the fish to discriminate between colour cues, while the other version required the fish to find a reward based on its position relative to the fish's starting point. By isolating the two different discriminatory cues between the two versions of the task, we were able to compare how these two types of cues influence learning speeds in two populations. On average across both versions, the two populations performed equally well. In other words, both populations were equally 'smart.' Moreover, there was no evidence that one version of the task was generally more difficult than the other. However, both populations showed cue-specific learning speeds-- they performed better on one version over the other -- but they excelled on different cues. Fish from one population (Navarro) performed better on the side discrimination version than on the colour discrimination version, while the opposite was

observed in the other population (Putah). These findings suggest that animals from different environments are primed to use different cues during learning, and therefore learn to make associations with certain cue types faster than others.

Fish from Putah Creek emerged significantly faster from a shelter (a behaviour often interpreted as 'boldness;' Brown and Braithwaite 2004; Burns 2008; Toms et al. 2010) than fish from the Navarro River. Previous studies have suggested that differences in boldness can contribute to differences in learning performance (e.g., DePasquale et al. 2014; Dugatkin and Alfieri 2003) because bolder, exploratory individuals are more likely to encounter relevant information needed to learn new contingencies (Carere and Locurto 2011; Sih and Del Giudice 2012). Despite these population level differences in 'boldness', the average performance of the two populations across the two versions of the discrimination task was the same. One possible explanation for this finding is that fish were always given an opportunity to find the reward regardless of how long it took them to approach the cups or if they initially went to the incorrect cup. This meant an association between the discriminatory cue and the reward could be made on every trial. Therefore, all individuals, independent of their boldness, had the same opportunity to learn during training. A previous study that compared stickleback populations similarly found that behaviours such as activity level and exploration were independent of learning rates when all individuals were allowed to receive the food reward after each trial (Park 2013).

The populations also differed in how quickly they acclimated to the experimental environment: on average, fish from Putah Creek completed pre-training in fewer days than fish from the Navarro River. Previous studies have suggested that individuals that have acclimated to the lab are better able to learn in a laboratory environment (Carere and Locurto 2011; Guillette et al. 2016). Even though they acclimated faster, fish from Putah Creek were not at an advantage in

this experiment because we used a criterion-based pre-training protocol in which fish were only allowed to move forward with the next step of training if they completed the previous step at their own pace. This meant that the fish were equally prepared to start training despite initial behavioural differences that might have otherwise influenced training (e.g., reluctance to explore, motivation or acclimation to the testing environment). Our approach – while time consuming – was effective and minimized biased sampling because over 96% (60/62) of the fish completed the training.

Despite similar average performance on the discrimination task, fish from Putah Creek did better on the colour version of the task, while fish from the Navarro River did better on the side version, which suggests that the populations are primed to associated certain cues with a food reward faster than others. While there is widespread evidence that animals rely on some cues over others during learning (Fiset et al. 2000; Garcia and Koelling 1966; Wheatcroft and Qvarnström 2015), and growing evidence that stickleback populations differ in the environmental cues they encode when foraging (Braithwaite and Girvan 2003; Girvan and Braithwaite 1998; Odling-Smee and Braithwaite 2003; Odling-Smee et al. 2008), our results build on these studies by showing that populations vary in how quickly they are able to associate different discriminatory cues with reward outcomes. Differences in learning speed can influence foraging efficiency and therefore fitness (Schoener 1971). Thus, this suggests differences in learning speeds may be a key source of cognitive variation between populations. This insight was only possible because cognitive performance was compared between two versions of a task that isolated the specific cues that were available to use during learning.

Thus the current study further contributes to the growing number of studies comparing cognitive performance among populations inhabiting different environments to understand the

evolution of cognitive mechanisms (e.g., Audet et al. 2015; Bond et al. 2007; Pravosudov and Clayton 2002; Tebbich and Teschke 2014). Our results are consistent with the hypothesis that ecology can drive adaptive cognitive processes, such that the local environment leads populations to more quickly associate specific cues with certain behavioural outcomes. While it is important to note that our sample size of two populations limits the ability of this study to identify specific ecological factors, our results are consistent with previous studies which suggested that habitat stability can drive differences in cognitive performance among stickleback populations (Girvan and Braithwaite 1998; Odling-Smee and Braithwaite 2003) For example, Girvan and Braithwaite (1998) suggested that sticklebacks from riverine environments are likely to experience high environmental variation due to water flow, and therefore might rely more on egocentric cues than visual cues, while sticklebacks from stable pond environments rely more on visual landmarks. Consistent with this hypothesis, fish from the Navarro River learned faster when the reward was associated with a specific turn sequence rather than with a visual cue. In contrast, fish from Putah Creek (subject to less dramatic changes) were faster to learn to follow a visual cue (colour). Further studies on replicate pond and river populations are needed in order to determine whether differences in habitat stability are responsible for driving the observed patterns, and it would be useful to know if the population-level variation has a genetic basis (as in (Pravosudov et al. 2012; Roth et al. 2011)). Additionally, the current study demonstrates that population variation in visual cue use extends to non-spatial variables such as colour. Elucidating the aspects (e.g., perception, learning and memory) of cognition that contribute to variation in cognitive performance will allow us to better understand the ecological factors that drive cognitive evolution. Here, we show that animals not only learn faster with certain types of cues over others, but that animals from different environments *differ* in the type of cues with

which they more readily form associations. Future studies that use a battery of tests to isolate different cognitive mechanisms are likely to further uncover the nuances of cognitive variation between populations and taxa. This is key to identify the ecological and evolutionary pressures that lead to fascinating variation in how animals process, learn and adapt to their environment.

ACKNOWLEDGEMENTS

We thank Emilee Johnson, Tyler Seal, Erika Carlson, and Amy Schneider for their help with data collection. We also would like to thank the Bell lab, as well as the editor and anonymous reviewers, for their comments to improve the manuscript. MB was supported by the National Science Foundation's Integrative Graduate Education and Research Traineeship program and funds from the University of Illinois Urbana-Champaign's School of Integrative Biology and Program in Ecology, Evolution, and Conservation Biology. This work was supported by NIH R01 GM082937 to AMB and NSF 1121980 to AMB.

COMPLIANCE WITH ETHICAL STANDARDS

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in this study involving animals were approved and in accordance with the ethical standards of the University of Illinois, Urbana Champaign (IACUC protocol # 15077). This article does not contain any studies with human participants performed by any of the authors.

FIGURES

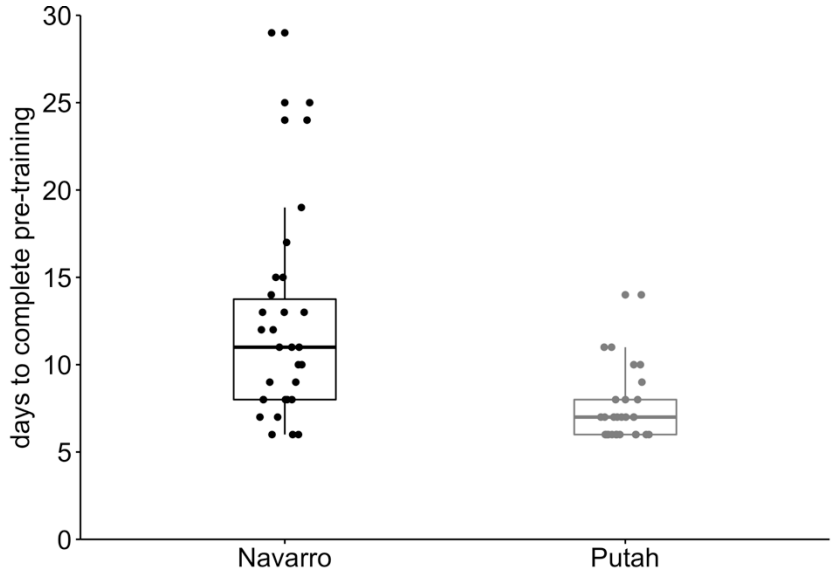


Figure 2.1. Population differences in *days to complete pretraining*. Each data point indicates a different individual, with median and IQR shown.

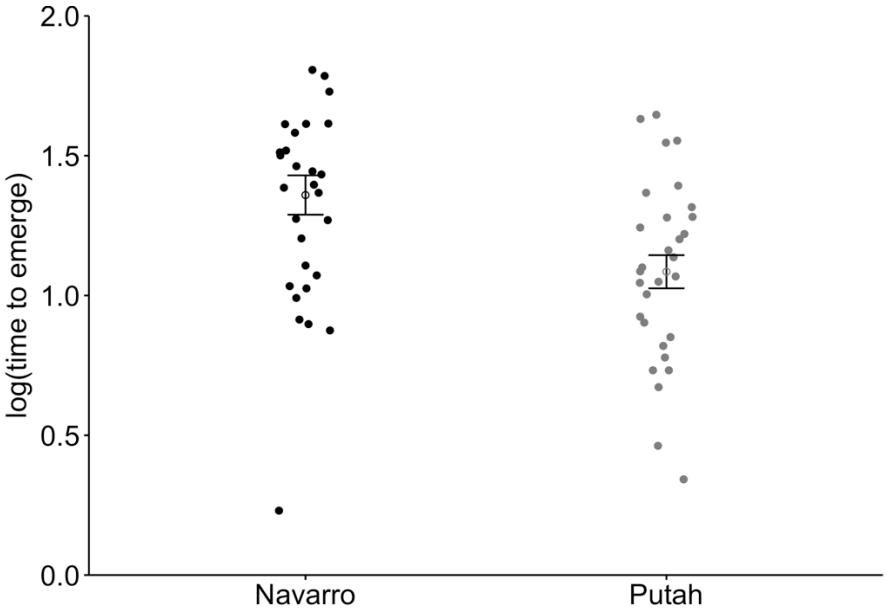


Figure 2.2. Population differences in average *time to emerge* (log-transformed) from starting shelter across the first training session (ten trials). Each data point indicates a different individual, with mean and SE shown.

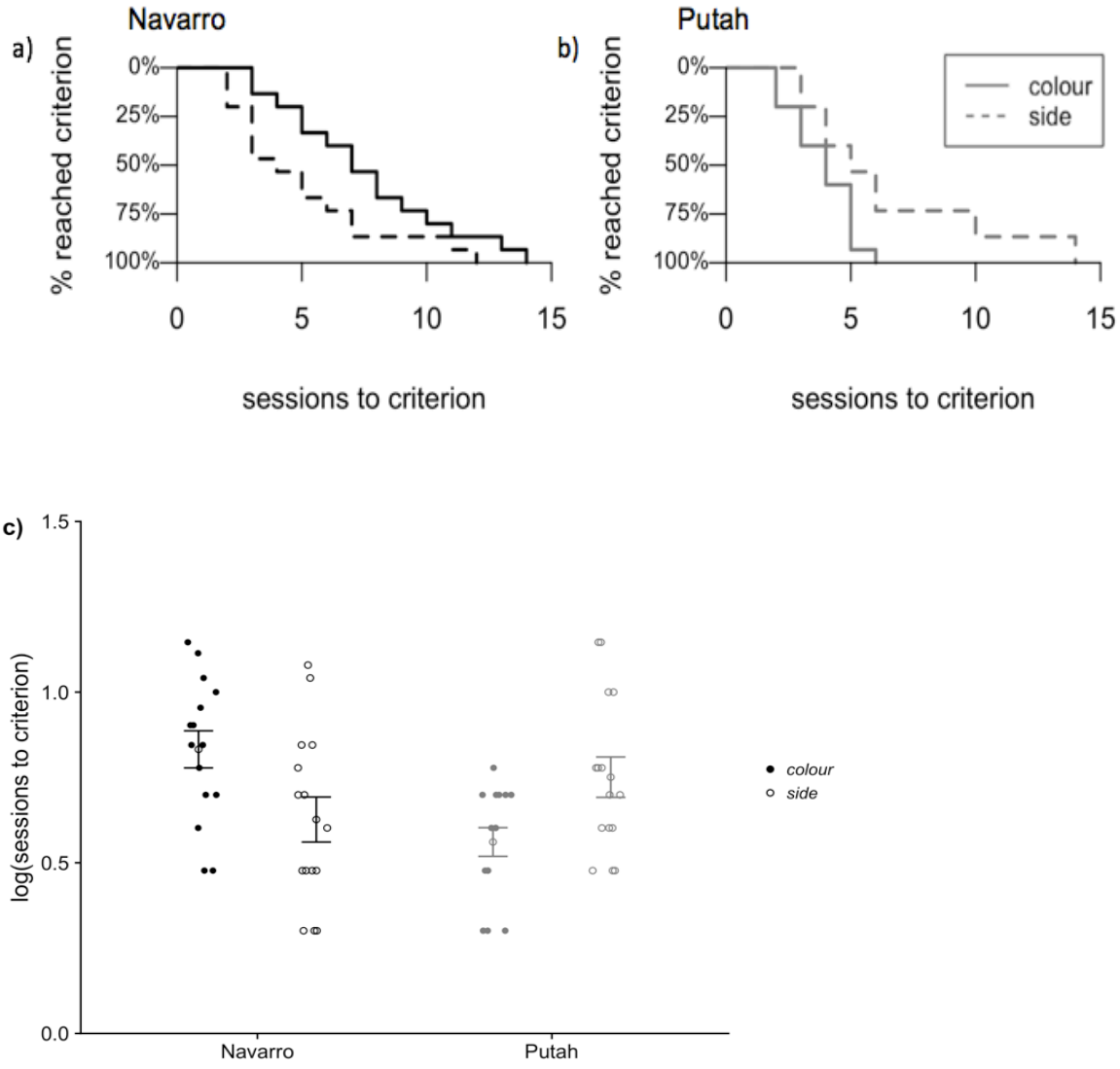


Figure 2.3. Learning performance of two populations on two versions of the discrimination task. The survival curves provide a side-by-side comparison of the rates at which fish reached criterion on each version of the task within the (a) Navarro (black) and (b) Putah (grey) populations. Note that the y-axis has been flipped to help indicate that as the curve descends it represents more fish reaching learning criterion. c) Comparison of the average performances on each version (colour – filled; side – open) within each population. Each data point indicates a different individual's log-transformed sessions to reach criterion, with mean and SE shown.

REFERENCES

Adriaenssens, B., & Johnsson, J. I. (2010). Shy trout grow faster: exploring links between personality and fitness-related traits in the wild. *Behavioral Ecology*, *22*(1), 135-143. (doi.org/10.1093/beheco/arq185)

Audet, J. N., Ducatez, S., & Lefebvre, L. (2015). The town bird and the country bird: problem solving and immunocompetence vary with urbanization. *Behavioral Ecology*, arv201. (doi: 10.1093/beheco/arv201)

Bell, A. M. (2005). Behavioural differences between individuals and two populations of stickleback (*Gasterosteus aculeatus*). *Journal of Evolutionary Biology*, *18*(2), 464-473. (doi: 10.1111/j.1420-9101.2004.00817.x)

Bell, M. A., & Foster, S. A. (1994). *The Evolutionary Biology of the Threespine Stickleback*. Oxford University Press.

Bensky, M. K., Paitz, R., Pereira, L., & Bell, A. M. (2017). Testing the predictions of coping styles theory in threespined sticklebacks. *Behavioural Processes*, *136*, 1-10. (doi: 10.1016/j.beproc.2016.12.011)

Bond AB, Kamil AC, Balda RP. (2007) Serial reversal learning and the evolution of behavioral flexibility in three species of North American corvids (*Gymnorhinus cyanocephalus, Nucifraga columbiana, Aphelocoma californica*). Journal of Comparative Psychology, 121(4):372. (doi: 10.1037/0735-7036.121.4.372)

Braithwaite, V. A., & Girvan, J. R. (2003). Use of water flow direction to provide spatial information in a small-scale orientation task. *Journal of Fish Biology*, *63*(s1), 74-83.

Brown, C., & Braithwaite, V. A. (2004). Size matters: a test of boldness in eight populations of the poeciliid *Brachyraphis episcopi*. *Animal Behaviour*, *68*(6), 1325-1329. (doi:10.1016/j.anbehav.2004.04.004)

Brydges, N. M., Heathcote, R. J., & Braithwaite, V. A. (2008). Habitat stability and predation pressure influence learning and memory in populations of three-spined sticklebacks. *Animal Behaviour*, *75*(3), 935-942.

Burns, J. G. (2008). The validity of three tests of temperament in guppies (Poecilia reticulata). *Journal of Comparative Psychology*, *122*(4), 344. (doi: 10.1037/0735-7036.122.4.344)

Carere, C., & Locurto, C. (2011). Interaction between animal personality and animal cognition. *Current Zoology*, *57*(4), 491-498. (doi: 10.1093/czoolo/57.4.491)

DePasquale, C., Wagner, T., Archard, G. A., Ferguson, B., & Braithwaite, V. A. (2014). Learning rate and temperament in a high predation risk environment. *Oecologia*, *176*(3), 661-667. (doi: 10.1007/s00442-014-3099-z)

Duffy, G. A., Pike, T. W., & Laland, K. N. (2009). Size-dependent directed social learning in nine-spined sticklebacks. *Animal Behaviour*, *78*(2), 371-375. (doi: 10.1016/j.anbehav.2009.05.015)

Dugatkin, L. A., & Alfieri, M. S. (2003). Boldness, behavioral inhibition and learning. *Ethology Ecology & Evolution*, *15*(1), 43-49. (doi: 10.1080/08927014.2003.9522689)

Feng, S., McGhee, K. E., & Bell, A. M. (2015). Effect of maternal predator exposure on the ability of stickleback offspring to generalize a learned colour–reward association. *Animal Behaviour*, *107*, 61-69. (doi: 10.1016/j.anbehav.2015.05.024)

Fiset, S., Gagnon, S., & Beaulieu, C. (2000). Spatial encoding of hidden objects in dogs (*Canis familiaris*). *Journal of Comparative Psychology*, *114*(4), 315. (doi: 10.1037/0735-7036.114.4.315)

Freas, C. A., LaDage, L. D., Roth, T. C., & Pravosudov, V. V. (2012). Elevation-related differences in memory and the hippocampus in mountain chickadees, Poecile gambeli. *Animal Behaviour*, *84*(1), 121-127. (doi: 10.1016/j.anbehav.2012.04.018)

Garcia, J., & Koelling, R. A. (1966). Relation of cue to consequence in avoidance learning. *Psychonomic science*, *4*(1), 123-124. (doi:10.3758/BF03342209)

Gill, A. B., & Hart, P. J. (1994). Feeding behaviour and prey choice of the threespine stickleback: the interacting effects of prey size, fish size and stomach fullness. *Animal Behaviour*, *47*(4), 921-932. (doi.org/10.1006/anbe.1994.1124)

Girvan, J. R., & Braithwaite, V. A. (1998). Population differences in spatial learning in three–spined sticklebacks. *Proceedings of the Royal Society of London B: Biological Sciences*, *265*(1399), 913-918. (doi: 10.1098/rspb.1998.0378)

Gottselig, J. M., Wasserman, E. A., & Young, M. E. (2001). Attentional trade-offs in pigeons learning to discriminate newly relevant visual stimulus dimensions. *Learning and Motivation*, *32*(2), 240-253. (doi:10.1006/lmot.2000.1081)

Guillette, L. M., Naguib, M., & Griffin, A. S. (2016). Individual differences in cognition and personality. *Behavioural Processes*, *134*, 1-3. (doi: 10.1016/j.beproc.2016.12.001)

Hampton, R. R., & Shettleworth, S. J. (1996). Hippocampal lesions impair memory for location but not color in passerine birds. *Behavioral Neuroscience,* 110(4), 831-835. (doi:10.1037/0735-7044.110.4.831)

Odling-Smee, L. C., Boughman, J. W., & Braithwaite, V. A. (2008). Sympatric species of threespine stickleback differ in their performance in a spatial learning task. *Behavioral Ecology and Sociobiology*, *62*(12), 1935-1945. (doi: 10.1007/s00265-008-0625-1)

Odling-Smee, L., & Braithwaite, V. A. (2003). The influence of habitat stability on landmark use during spatial learning in the three-spined stickleback. *Animal Behaviour*, *65*(4), 701-707. (doi: 10.1006/anbe.2003.2082)

Park, P. J. (2013). Spatial learning ability of the threespine stickleback (*Gasterosteus aculeatus*) in relation to inferred ecology and ancestry. *Evolutionary Ecology Research*, *15*(2), 213-239.

Pravosudov, V. V., & Clayton, N. S. (2002). A test of the adaptive specialization hypothesis: population differences in caching, memory, and the hippocampus in black-capped chickadees (*Poecile atricapilla*). *Behavioral Neuroscience*, *116*(4), 515.

Pravosudov, V. V., Roth, T. C., Forister, M. L., Ladage, L. D., Burg, T. M., Braun, M. J., & Davidson, B. S. (2012). Population genetic structure and its implications for adaptive variation in memory and the hippocampus on a continental scale in food-caching black-capped chickadees. *Molecular Ecology*, *21*(18), 4486-4497. (doi: 10.1111/j.1365-294X.2012.05721.x)

R Core Team (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Roche, D. P., McGhee, K. E., & Bell, A. M. (2012). Maternal predator-exposure has lifelong consequences for offspring learning in threespined sticklebacks. *Biology Letters*, *8*(6), 932-935. (doi: 10.1098/rsbl.2012.0685)

Rodriguez, F., Duran, E., Vargas, J. P., Torres, B., & Salas, C. (1994). Performance of goldfish trained in allocentric and egocentric maze procedures suggests the presence of a cognitive mapping system in fishes. *Learning & behavior*, *22*(4), 409-420.

Rodríguez, F., López, J. C., Vargas, J. P., Gómez, Y., Broglio, C., & Salas, C. (2002). Conservation of spatial memory function in the pallial forebrain of reptiles and ray-finned fishes. *Journal of Neuroscience*, *22*(7), 2894-2903.

Roth, T. C., & Pravosudov, V. V. (2009). Hippocampal volumes and neuron numbers increase along a gradient of environmental harshness: a large-scale comparison. *Proceedings of the Royal Society of London B: Biological Sciences*, *276*(1656), 401-405. (doi: 10.1098/rspb.2008.1184)

Roth, T. C., LaDage, L. D., Freas, C. A., & Pravosudov, V. V. (2011). Variation in memory and the hippocampus across populations from different climates: a common garden approach. *Proceedings of the Royal Society of London B: Biological Sciences*, rspb20111020. (doi: 10.1098/rspb.2011.1020)

Schoener, T. W. (1971). Theory of feeding strategies. *Annual review of ecology and systematics*, *2*(1), 369-404. (doi: 10.1146/annurev.es.02.110171.002101)

Sih, A., & Del Giudice, M. (2012). Linking behavioural syndromes and cognition: a behavioural ecology perspective. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, *367*(1603), 2762-2772. (doi: 10.1098/rstb.2012.0216)

Tebbich, S., & Teschke, I. (2014). Coping with uncertainty: woodpecker finches (*Cactospiza pallida*) from an unpredictable habitat are more flexible than birds from a stable habitat. *PloS One*, *9*(3), e91718. (doi: 10.1371/journal.pone.0091718)

Therneau T (2015). _A Package for Survival Analysis in S_. version 2.38, <URL:https://CRAN.R-project.org/package=survival>.

Toms, C. N., Echevarria, D. J., & Jouandot, D. J. (2010). A methodological review of personality-related studies in fish: focus on the shy-bold axis of behavior. *International Journal of Comparative Psychology*, *23*(1).

Visser, M. E., Schaper, S. V., Holleman, L. J., Dawson, A., Sharp, P., Gienapp, P., & Caro, S. P. (2011). Genetic variation in cue sensitivity involved in avian timing of reproduction. *Functional Ecology*, *25*(4), 868-877. (doi: 10.1111/j.1365-2435.2011.01844.x)

Wheatcroft, D., & Qvarnström, A. (2015). A blueprint for vocal learning: auditory predispositions from brains to genomes. *Biology Letters*, *11*(8), 20150155. (doi: 10.1098/rsbl.2015.0155)

Wickham H. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York, 2009.

Wilson, A. D., & Godin, J. G. J. (2009). Boldness and behavioral syndromes in the bluegill sunfish, Lepomis macrochirus. *Behavioral Ecology*, arp018. (doi: 10.1093/beheco/arp018)

CHAPTER 3: PREDICTORS OF INDIVIDUAL VARIATION IN REVERSAL LEARNING PERFORMANCE IN THREESPINED STICKLEBACKS

ABSTRACT

Behavioral flexibility refers to the ability of an animal to switch from a previously reinforced behavior pattern in response to a changing environment and might be important for animals in temporally variable environments. Performance on a reversal learning task is often used as a measure of behavioral flexibility, but variation in reversal learning performance could reflect multiple processes. Here we examine how individual differences in behavior might drive variation in reversal learning performance in threespined sticklebacks. Female stickleback were measured in a series of three behavioral assays: response to a novel object to measure environmental sensitivity, willingness to emerge into a novel environment to measure boldness, and response to a barrier to measure inhibitory control. Then, the same individuals were assessed in a two-way discrimination learning and reversal task. Time to criterion in the reversal learning task was not correlated with any of the behaviors measured. However, individuals that made fewer mistakes during early reversal learning trials were more sensitive to a novel object, less bold, less persistent in the barrier detour task, and performed worse during initial learning. These results highlight the utility of dissecting the steps of reversal learning in order to understand the underlying causes of variation in behavioral flexibility.

INTRODUCTION

As concerns rise about how climate and anthropogenic change will continue to impact global biodiversity and ecosystem stability, there is increasing interest in elucidating the traits that allow some organisms to respond adaptively and thrive in a changing environment. Behavioral flexibility is a type of phenotypic plasticity that can influence how animals cope with environmental change. While the term "behavioral flexibility" has been used to refer to a wide range of phenomenon, including problem-solving and innovation (Audet and Lefebvre 2017), here we specifically focus on the ability of an animal to switch away from a previously reinforced behavior pattern in response to a change in the stimulus environment (see Stamps 2016). Animals that are sensitive to changes in the environment, and phenotypically plastic enough to respond adaptively to those changes, might have higher fitness across temporal and/or spatial scales (Wolf et al. 2008). For example, behaviorally flexible animals might be better able to identify new food sources when previous sites become scarce or dangerous (Dill 1983). Additionally, flexibility can improve fitness if it allows individuals to identify opportunities to gain access to mates (Shine et al. 2005a,b), or adaptively respond to predation threats to offspring (Lima 2009). Therefore flexibility has the potential to influence evolutionary outcomes (Foster and Sih 2013; Ghalambor et al. 2007; West-Eberhard 2003).

Behavioral flexibility is often measured via reversal learning paradigms. Animals are trained to discriminate between two or more variations of a cue (e.g., color) by associating one particular cue (e.g. the color blue) with a reward (e.g. food). After a predetermined number of trials or after the subject has reached a predetermined criterion, the reward is moved to a different location on subsequent trials and reversal learning performance (behavioral flexibility)

is often assessed by some measure of how quickly the individual learns the new association. However, it is unclear to what extent individual differences in behavior might be associated with different aspects of reversal learning performance, or if these patterns might differ based on when you examine performance. As a result, it is often unclear how variation in performance might generalize to contexts outside of the learning environment.

An animal might perform well in a reversal learning paradigm for several different reasons (den Ouden et al. 2013; Izquierdo et al. 2017). Particularly sensitive individuals might be more attuned to changes in environmental cues and more attentive to unexpected outcomes (Aron and Aron 1997; Aron et al. 2012; Chudasama et al. 2003; Schoenbaum et al. 2009), and therefore excel on a reversal learning task. For example, mice that performed well on a reversal learning paradigm were more sensitive to changes in the environment: they modulated their aggression toward males versus females, and changed their behavior in response to changes in light/dark cycle (Benus et al. 1990). Environmental sensitivity may manifest as an increased stress response to novel objects (i.e., neophobia), but then also lead to improved attention to changes within familiar contexts (Bolhuis et al. 2004; Bebus et al. 2016). For example, lines of trout that had been selected for high cortisol stress responsiveness were highly attentive to changes in the location of food patches (de Lourdes Ruiz-Gomez et al. 2011).

Another possibility is that variation in reversal learning performance reflects variation along the shy-bold continuum. Bold individuals, for example, might be more likely to learn new associations because they are more willing to take risks to acquire new information (Carere and Locurto 2011; Dougherty and Guillette 2018; Griffen et al. 2015; Sih and Del Giudice 2012).

Finally, another mechanism that might lead to differences in reversal learning performance is inhibitory control: some individuals might be better able to inhibit a response that

has been previously rewarded once that response is no longer effective, and therefore quickly learn a new association (Izquierdo and Jentsch 2012; Roberts and Wallis 2000). That is, flexible individuals might have relatively high inhibitory control, while persistent individuals become routinized and perseverate because they can't 'turn off' a learned association.

Complicating matters further is that reversal learning itself comprises a series of different steps, all of which could contribute to overall reversal learning performance as assessed by, e.g. time to criterion (Boulougouris et al. 2008). For example, the individual must first notice that the contingency has changed. Then, the individual has to inhibit a previously-learned response. Then, the individual must forget the previously established behavior pattern (Tello-Ramos et al. 2019). Finally, the individual has to learn the new contingency. It is plausible that there is individual variation in processes influencing all of these steps, therefore overall reversal learning performance could reflect multiple, heterogeneous causes.

In the current study we break down reversal learning performance as a measure of behavioral flexibility by measuring the same individuals on a series of assays designed to measure environmental sensitivity (response to a novel object), boldness (latency to emerge from a refuge), inhibitory control (reaction to a barrier), and discrimination and reversal learning performance on a color discrimination task in a fixed order. We then dissect the steps of reversal learning performance to distinguish the initial response to a change in contingency from the acquisition of a new association (as assessed by time to criterion). This allowed us to investigate whether individual differences in environmental sensitivity, boldness and inhibitory control were correlated with variation in certain steps of reversal learning but not others. For this study we used the threespined stickleback, an important model for examining natural intra-specific

variation in behavior and cognition, including behavioral flexibility (Bensky et al. 2017; Rystrom et al. 2019).

MATERIALS & METHODS

Subjects

Adult sticklebacks were collected from Putah Creek, CA in summer 2016. Fish were transported to the University of Illinois Urbana-Champaign where they were housed in 37.85L 53 x 33 x 24 cm tanks in groups of ten to fifteen fish. Fish were housed in 20 degrees Celsius water and on a summer (16L: 8D) photoperiod. A recirculating flow-through system consisting of a series of particulate, biological, and UV filters (Aquaneering, San Diego, USA) was used to clean the water. 10% of the water volume in the tanks was replaced each day. The fish were fed a mixed diet consisting of frozen bloodworms, frozen brine shrimp, and frozen mysis shrimp ad lib each day. Once the experiment began, fish were only fed during the feeding and learning trials in order to maintain motivation.

Wild-caught adult females (n=60) were used in the experiment. Females were reproductively mature but were not gravid during the experiment (average length = 48.9 mm, length standard deviation = 4.7 mm, average mass = 1.54 g, mass standard deviation = 0.38 g). Once a female started the experiment, she was housed individually in a 26.5 L 36 x 33 x 24 cm tank. The tank bottom was divided into thirds, and the floor of the outer two-thirds were lined with gravel. A plastic plant was placed at the center of the middle third of the tank. Individuals had visual access to fish in adjacent tanks between testing trials.

To ensure that individuals were acclimated before beginning the experiment, we measured how quickly the fish ate during a feeding trial. Once a day, the plastic plant was

removed from the tank, and plastic blinders were used to block all sides of the tank. A mirror was positioned above the tank to allow experimenters to observe the fish with minimal disruption. This same procedure was followed during all of the different assays, regardless of if the assay was conducted in the individual's home tank or in a separate testing tank. A glass petri dish (60W x 15H mm; Corning Inc, Corning, USA) was then placed in the center of the tank with 10 bloodworms placed inside and the foraging behavior of the fish was recorded for ten minutes. Once the fish ate, the plant was returned to the tank and the blinders were removed. We inferred that an individual was acclimated and motivated after the individual ate at least 8 out of 10 worms within 10 minutes on three consecutive days; the individual was tested in the novel object assay the following morning.

Response to a novel object

The purpose of this assay was to assess individual differences in sensitivity to the presence of a novel object in their home environment. A camcorder (JVC Everio HD Hard Dish Camcorder Model No: GZ-HD40U) was used to record a top down view of the trial via the mirror positioned above the tank. A perforated tank separator was placed into the tank to ensure that the fish could not access the back of the tank, which would have put it out of the view of the camera. A circular blind was used to corral the individual into the middle third of the tank, where the individual was undisturbed for 5 minutes. While the fish was in the blind, a toy plastic lion (10L x 7H cm; TERRA by Battat, Montreal, Canada) was placed into either the left or right third of the tank at random (Figure 3.1a). This toy was selected as it was never seen by the fish previously, the shape is presumed to have no evolutionary relevance, and the colors were neutral.

After 5 minutes, the blind was removed, and the trial began. We recorded the time it took the individual to orient towards the novel object (i.e., nose pointed directly towards the novel object), the time it took the individual to approach the novel object (i.e., head within one body length of the novel object), the time the individual spent within the third of the tank that contained the novel object, as well as the time the individual spent both within this third and oriented towards the novel object for five minutes. Upon completion of the task, the plant was returned to the tank and the blinders were removed.

Response to a barrier and boldness

The purpose of the barrier task was to assess individual differences in inhibitory control. Individuals were trained to follow a search pattern in order to obtain a food reward. Then, they were confronted by a transparent barrier separating them from the food reward. In order to obtain the food reward, the fish had to swim around the barrier. An individual that followed the prepotent search pattern established during training entered the barrier space and hit the barrier at its nearest point to the food reward, hereafter referred to as the "barrier apex" (Figure 3.1b). We interpret greater time spent at the "barrier apex" as persistence with the prepotent response, or low inhibitory control. Training for the barrier task involved placing an individual in a refuge, from which they could emerge voluntarily. Emergence time from a refuge is often interpreted as a measure of "boldness" (Wilson and Godin 2009), therefore we also recorded the time to emerge from the refuge on the first training trial as a proxy for boldness.

Training for the barrier detour task started the afternoon following the novel object assay. The goal of this training was to both establish food motivation within a novel space, as well as establish a prepotent response of leaving a shelter to directly approach and eat a food reward.

Establishing this direct food search pattern was important for then interpreting behavioral responses during the barrier task. All training occurred in a separate testing tank that was the same dimensions as the individual's home tank except that it did not contain gravel.

Individuals were trained for one session per day, and each session comprised of four trials. To begin the session, the individual was gently scooped with a white cup from their home tank and transferred to an opaque shelter that was then placed into the back-center of the testing tank and the individual was allowed to acclimate for three minutes. A glass petri dish with a single bloodworm was placed in the center of the test tank (~7 cm from the entrance of the shelter). To start the trial, the observer removed a cork from the side of the shelter and the latency for the individual to emerge from the shelter and to eat the worm was recorded. If the individual ate the reward, the observer gently scooped up the fish and placed the fish back into the shelter in preparation for the next trial. If the fish did not emerge from the shelter within ten minutes after the cork was pulled or eat within five minutes after emergence, the observer recorded the maximum times for these behaviors, removed the food reward and gently poured the fish out of the shelter if necessary. Between trials fish were allowed to reacclimate to the shelter for 2 minutes. After the fourth trial of the day the fish were returned to their home tanks.

Training for the barrier task was criterion based, and was continued until the criterion was reached. In order to move on to the barrier task, the individual had to emerge from the shelter within 10 minutes and directly approach and eat the food reward within five seconds on three out of the four trials. The one failed attempt could not be on the fourth trial. This criterion was used to ensure that the fish would be motivated throughout the four trials; three fish did not meet this criterion and were omitted from the study.

After reaching criterion, the individual moved on to the barrier detour task the following day. This task also consisted of four trials. In order to reinforce the direct search pattern, the first three trials were exactly the same as the training trials. On the fourth trial a transparent semi-circular barrier was placed between the shelter and food reward. The opening into the barrier was positioned directly in front of the entrance to the shelter. After removing the cork the individual was allowed 30 minutes to emerge from the shelter, navigate around the barrier and eat the food reward. All of the fish ate within 30 minutes. We recorded the duration of the first bout at the "apex" (where bout is repeated contact with the barrier apex with no break for longer than five seconds), total time at the apex and the latency to move away from the apex to swim around the ends of the barrier. Upon completion of the fourth trial, the individual was returned to its individual home tank.

Initial and reversal learning

We measured individual differences in initial and reversal learning performance using a two-way discrimination task, as in Bensky et al. 2017. Each fish was trained individually in the same tank that was used for the barrier task. Training began the day following the barrier task, and the first phase was aimed at training the fish to search two possible locations for a food reward. During this phase fish were trained to emerge from a shelter and search two colorless cups that each had a portion of their sides removed to allow access into the cups; the food was accessible in one cup while in the other cup, the food was blocked with tape. Fish received two trials a day (i.e., the rewarded cup was on each side once), and met criterion once they readily ate from the cups on both trials on three consecutive days. One fish was suspended at this stage due to lack of motivation.

During the discrimination training, a blue and yellow cup were presented on each trial, and one of the cups was rewarded with food. Individuals received one session a day; each session consisted of 10 trials. Between trials, fish were gently scooped back into the starting shelter and given two minutes to rest. Each fish received 10 trials before the next fish was trained. All fish were initially trained to associate the blue cup with a food reward until that individual reached the training criterion, which was two consecutive sessions of choosing the correct cup on at least 8 out of 10 trials. The openings of the cups were faced towards the front of the tank (away from the starting shelter), and the fish's choice was determined based on which of the choice zones it entered first while orienting towards the center of the corresponding cup (Figure 3.1c). Three fish were suspended during this initial training stage due to lack of motivation (i.e., did not make a choice on three trials per session for consecutive sessions).

The day after criterion was met, the individual received an additional trial with the rewarded blue cup to ensure that they were still following the initial association, and then the reversal phase begun. During the reversal learning phase, the food was always placed into the yellow cup and again each individual was trained until criterion. There is no evidence of a color bias for blue or yellow in this population (Bensky and Bell 2018).

To measure initial learning performance, we recorded the number of sessions it took each individual to reach criterion. To measure individual variation in behavioral flexibility via reversal learning, we broke down the steps of reversal learning performance into two components: 1) the individual's behavior immediately after the rewarded cup was switched from blue to yellow (i.e. behavior during the first trial of reversal learning, as well as performance across the first 10 reversal trials) and 2) acquisition of the new association between the yellow cup and the reward, i.e. time to criterion during the reversal phase.

While all of the individuals initially searched the blue cup on the first trial of reversal learning (as would be expected if the initial association with blue was learned), there was variation among individuals in the number of times they visited the blue cup before searching the yellow cup. Roughly half the individuals only searched the blue cup once before swimming over to the yellow cup, while the other half of the individuals searched the blue cup repeatedly. Recent findings suggesting that documenting "microbehaviors" within paradigm performance can be fruitful for identifying behavioral correlates of variation in cognitive processes (Chow et al. 2017). Therefore, we used a binary-based score to indicate whether or not individuals persisted on the blue cup, where individuals that searched the blue cup only ,once were scored "0" while individuals that revisited the blue cup more than once were scored "1".

Statistical Analysis

53 individuals completed the experiment. Positively-skewed duration and latency variables were log-transformed to improve normality. All analysis was done using R 3.4.2 (http://www.r-project.org/). "Boldness" was based on a single duration measure from the first barrier task training trial. For ease of interpretation, we multiplied the log-transformed emergence time for the first trial by negative one so that greater values indicate shorter emergence times, i.e. greater boldness. To reduce the number of variables involved for the other two assays, we performed two separate principle component analyses (PCA) on the correlation matrices of the variables measured in the environmental sensitivity and barrier detour task, respectively (R Core Team 2016; package = "factoextra"; function = "prcomp"). $PC_{\text{novel object}}$ explained 74.5% of the variation in four variables recorded in the response to a novel object test (Table 3.1a), where greater $PC1_{\text{novel object}}$ scores indicate longer times to orient and approach the

novel object, and less time spent near and oriented towards the novel object. $PC1_{barrier}$ explained 77.2% of the variation in three variables measured in the barrier task (Table 3.1b), where greater $PC1_{barrier}$ scores indicate longer first bouts and total durations at the apex, as well as longer times to first attempt an alternative route that required moving away from the apex.

Given previous findings showing correlations between body size, behavior (e.g., emergence times: Brown and Braithewaite, 2004), and learning (e.g., Amiel et al., 2014), we tested for significant associations between body length and behavior to test if body size would need to be statistically controlled in downstream models. Linear regression models were generated to test for the effect of body size on the PC scores. Sessions to criterion and number of correct trials over the first reversal session were treated as count data, therefore generalized linear models were used to measure the effect of size on these variables. To control for overdispersion, a negative binomial distribution was used for these models. A logistic regression model was used for our binary persistence variable from the first reversal trial. Larger fish took longer to reach criterion on initial learning ($\beta = -0.042$, $z = -2.153$, $p = 0.0313$, $n=53$), but standard length was not related to any of the other variables in this study (Table C.1). Therefore, standard length was included as a covariate in models involving sessions to reach criterion on initial learning, but was not included in other models.

To examine correlations among the three behavioral variables we computed pairwise Pearson correlation coefficients. To examine the relationships among the behavioral variables and the learning measures, we used generalized linear modeling which allowed us to take into account body length as a covariate, and incorporate binomial and negative binomial distributions where appropriate. Finally, generalized linear models were used to test for the relationship between initial and reversal learning performance. In this case, because length was correlated

with initial learning, individual body length was first regressed onto time to initial criterion and the resulting residuals were then used to predict reversal learning performance.

RESULTS

Correlations among behavior in the novel object assay, emergence assay and barrier task

To investigate the extent that the measured behavioral traits corresponded with one another across contexts, we examined the pairwise correlations between the three traits. There was a trend for more sensitive individuals (i.e. greater $PC1_{novel\ object}$; individuals which slowly oriented to and approached the novel object and which spent little time near the novel object) to take longer to emerge from the shelter ($r = -0.218$, $p = 0.1176$, $n=53$). The individuals that spent more time at the barrier apex (i.e. greater $PC1_{barrier}$) were also the individuals that emerged relatively quickly from the shelter ($r = 0.295$, $p = 0.0317$, $n=53$). There was a trend for more sensitive individuals, i.e. greater $PC1_{novel\ object}$, to spend less time at the barrier apex, i.e. greater $PC1_{barrier}$ ($r = -0.265$, $p = 0.057$, $n=53$).

Learning

To assess general learning patterns between the initial and reversal learning phases, we looked at how fish performed during the first session of each phase and the average number of days it took to reach criterion. At the beginning of the learning trials, individuals chose the blue and yellow cups at relatively equal frequency. Performance improved with training as individuals increasingly chose the blue cup across sessions. On average, individuals reached criterion after 5.28 +/- 4.17 sd sessions (range = 2-18 sessions; Figure 3.2a).

In contrast, individuals started the reversal learning phase performing significantly below chance levels (average proportion correct = 0.174, sd = 0.169, $z = 4.747$, $p < 0.0001$; Figure 3.2b), which suggests that in general the fish persisted on the previously rewarded association during the early stages of reversal learning. On average, individuals took 8.08 +/- 3.3 sd sessions (range = 4-18 sessions) to reach criterion during reversal learning. The average learning speeds for both phases were very similar to the those seen in Bensky et al. (2017).

Correlations among behavior in the novel object assay, emergence assay and barrier task and learning

Individuals that were less sensitive to the novel object (smaller $PC1_{\text{novel object}}$) and which spent more time at the barrier apex (greater $PC1_{\text{barrier})}$) learned the initial discrimination faster ($PC1_{\text{novel object}}$: $\beta = 0.162$, $z = 3.37$, $p = 0.0007$, n=53; $PC1_{\text{barrier}}$: $\beta = -0.196$, $z = -3.732$, $p = 0.0002$, n=53). We did not detect a relationship between sessions to criterion on initial learning and emergence time ($\beta = -0.075$, $z = -1.102$, $p = 0.27$, n=53).

We did not detect any significant correlations between any of the behaviors from the behavioral assays and sessions to criterion during reversal learning ($PC1_{\text{novel object}}$: $\beta = -0.038$, $z = 1.173$, $p = 0.241$, n=53; $PC1_{\text{barrier}}$: $\beta = -0.043$, $z = -1.173$, $p = 0.241$, n=53; emergence time: $\beta = 0.0068$, $z = 0.174$, $p = 0.862$, n=53).

However, behavior in the behavioral assays were correlated with aspects of *early* reversal performance. Specifically, individuals that were more sensitive to the novel object chose the correct cup more often during the first 10 reversal trials compared to less sensitive individuals ($PC1_{\text{novel object}}$: $\beta = 0.234$, $z = 3.714$, $p = 0.0002$, n=53, Figure 3.3). We did not detect significant relationships between first reversal session performance and behavior during the barrier task or

emergence time ($PC1_{barrier}$: β = -0.1048, z = -1.251, p = 0.211, n=53; boldness: β = -0.147, z = -1.574, p = 0.12, n=53). However, when the rewarded cup was switched from blue to yellow, some individuals repeatedly visited the previously rewarded blue cup, and those individuals that repeatedly visited the blue cup were the ones that spent more time at the barrier apex (β = 0.4849, z = 2.265, p = 0.024, n=53, Figure 3.4a) and were more bold (emerged faster) (β = 0.556, z = 2.36, p = 0.018, n=53, Figure 3.4b).

Initial and reversal learning

Sessions to criterion on initial learning was not associated with sessions to criterion on reversal learning (β = -0.0217, z = -0.304, p = 0.761), or the probability that an individual would perseverate on the initially rewarded blue cup (β = -0.2262, z = -0.59, p = 0.555). However, fish that took longer to reach criterion during the initial learning phase had higher performance (more correct trials) during the first 10 reversal trials (β = 0.3308, z = 2.324, p = 0.0201, Figure 3.5).

DISCUSSION

Reversal learning is a common paradigm used to measure behavioral flexibility, but it is still unclear how performance differences might generalize to different behavioral contexts. It is also unclear whether early or long-term learning responses are a better indicator of how quickly an animal will respond to a change in its environment. Thus our understanding of the biological relevance of reversal learning performance variation is still limited. In the current study, the way that individuals immediately reacted to a change in the learning environment (i.e. their behavior after the rewarded cup was switched from blue to yellow) was related to their response to a novel

object, boldness, response to a barrier and initial learning performance. Specifically, individuals that were relatively sensitive to the novel object were faster to abandon the search pattern that had been reinforced during the initial learning phase, and performed better across the first ten trials of the reversal learning phase compared to their less sensitive counterparts. In addition, relatively bold individuals, and those that spent more time at the barrier apex, were more likely to return to the previously rewarded location multiple times within the first reversal trial before searching for an alternative location. Finally, there was a trade-off between initial learning performance and reversal learning performance: when the food was switched from the blue to the yellow cup, those that learned the initial discrimination faster tended to perform worse immediately following the change in reward location.

While we could partially explain individual variation in early reversal learning performance by understanding individual differences in reaction to a novel object (sensitivity), reaction to a barrier (inhibitory control), latency to emerge from a refuge (boldness) and initial learning performance, behaviors in those contexts were not correlated with the most commonly-used metric of reversal learning performance: time to criterion. We speculate that early reversal learning performance reflects the ability of an animal to switch away from a previously learned behavior. In contrast, time to criterion is more heterogenous; it reflects both the ability of an animal to switch away from a previously learned behavior *and* the ability of an animal to establish a new rewarded pattern, which might help explain the failure to detect correlations between time to criterion and individual differences in behavior in other contexts. Interestingly, other studies have shown that different steps of reversal learning are chemically dissociable in the brain. For example, the dopaminergic system is involved in perseveration on previously reinforced patterns, perhaps via reward-based effects (Cools et al. 2009), while serotonin

influences the rate at which individuals shift away from stimuli associated with punishment or negative outcomes (Chamberlain et al. 2006, den Ouden et al. 2013).

An important mechanism that may also contribute to the variation in reversal learning performance observed in this study is memory (Tello-Ramos et al. 2019). That is, some individuals might have formed stronger memories than others, and their strong memories might have interfered with their ability to make new associations (i.e., proactive interference; Anderson and Neely 1996). Trade-offs between memory and behavioral flexibility have been documented at both the inter- and intraspecific level (Croston et al. 2017; Hampton et al. 1998; Lewis and Kamil 2006) so this is a promising area for future work.

In summary, we found that individual differences in the reaction to a novel object, boldness, inhibitory control and initial learning predict variation in some aspects of reversal learning performance in threespined stickleback. Importantly, we could explain variation in aspects of *early* learning performance, when individuals were just starting to switch away from a previously rewarded behavioral pattern, but not time to criterion, a metric commonly used in reversal learning paradigms. The fact that behavioral correlations are only found with early reversal learning responses, and that we only see a trade-off with initial learning and early reversal behavior, lends support to the idea that behavioral flexibility as a cognitive trait may be more about switching away from learned behaviors versus learning new associations. This suggests we may want to focus more of our attention on early reversal learning responses in order to better our understanding of learning trade-offs and develope hypotheses about how performance in the lab may generalize to more natural contexts. As the cognitive ecology field continues to grow, and more studies focus on inter- and intra-individual cognitive variation in a growing list of species, there is an increasing need to examine the accuracy and validity of the

measurements used to purportedly measure cognitive processes of interest (Boogert et al. 2018). Such research will help us better understand which aspects of cognitive performance generalize across behavioral contexts (Cauchoix et al. 2018; Völter et al. 2018) in order to help interpret cognitive variation.

ACKNOWLEDGEMENTS

We are grateful to the members of the Bell lab for their help throughout this project. We would also like to thank Sean Ehlman and Rebecca Halpin of the University of California Davis for their help with fish collection at the Putah Creek Reserve. Financial support was provided by the National Science Foundation's Integrative Graduate Education and Research Traineeship program and by the University of Illinois. This material is based upon work supported by the National Science Foundation under Grant No. IOS 1121980, by the National Institutes of Health under award number 2R01GM082937-06A1.

COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflict of interest. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in this study involving animals were in accordance with the ethical standards of the University of Illinois, Urbana Champaign (IACUC protocol #15077). This article does not contain any studies with human participants performed by any of the authors.

TABLE AND FIGURES

a)

Response to a novel object (log-transformed)	**$PC1_{novel\ object}$ Loadings** **Eigenvalue: 2.98 ; %variance: 74.5%**
time to orient	0.771
time to approach	0.917
time spent in the zone containing the novel object (5 minute)	-0.893
time spent in the zone containing the novel object and oriented toward the novel object	-0.863

b)

Barrier detour task (log-transformed)	**$PC1_{barrier}$ Loadings** **Eigenvalue: 2.32; %variance: 77.2%**
first apex bout duration	0.911
total time at apex	0.937
time to first attempt towards barrier edge	0.780

Table 3.1. Loadings on the first principle components for the a) novel object assay, and b) barrier detour task. All behaviors were duration measures and were log-transformed to normalize the data prior to analysis.

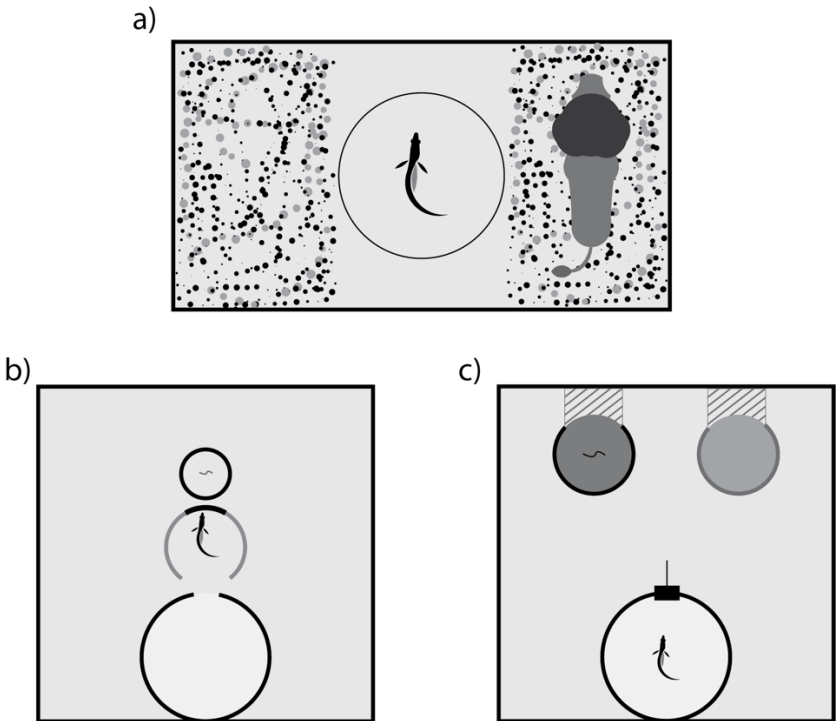


Figure 3.1. Visual description of the tanks used for behavioral observations. a) The novel object assay (top-down view). An individual was placed in a circular blind in the center third of the home tank. A novel object (toy lion) was then placed at one end of the tank. After 5 minutes, the blind was removed, and the individual's behavior was monitored. **b) The barrier detour task.** Fish were trained to find food upon leaving a shelter. Then, a transparent barrier was placed between the shelter and the food. Upon emerging from the starting shelter, the individual's interaction with the transparent barrier was recorded. The black region of the barrier denotes the "barrier apex". **C) The color discrimination task.** The individual began each trial in the starting shelter. Once the cork was removed and the individual emerged from the shelter, the fish's behavior was observed. In particular, we noted whether the fish entered the choice zone (indicated by stripes) of the blue or yellow cup while orienting towards the chosen cup.

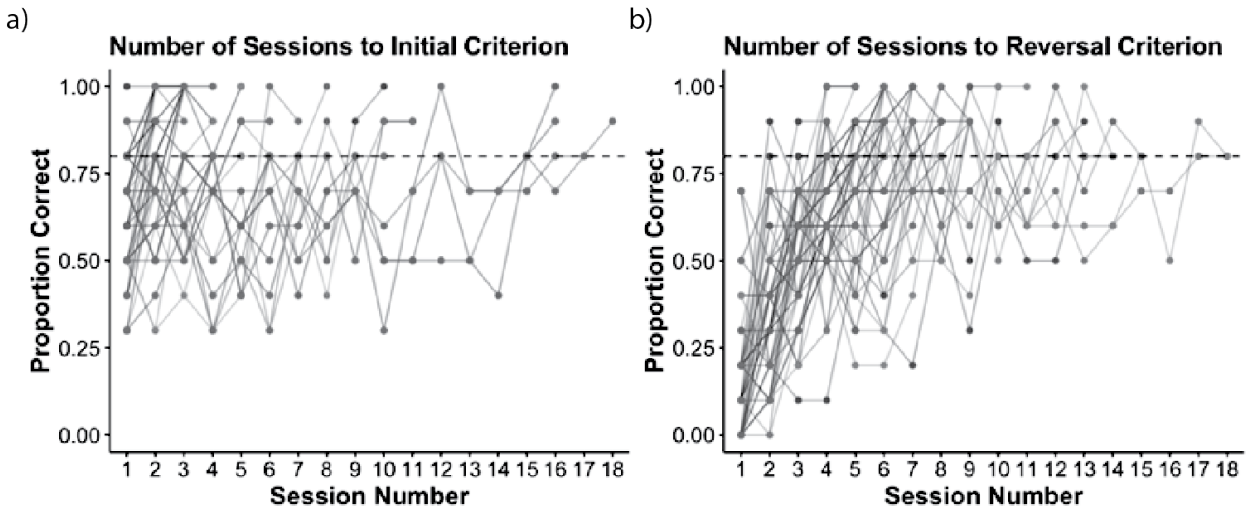


Figure 3.2. Individual learning curves from a) initial learning, and b) reversal learning discrimination phases. Lines represent the performance of individuals across sessions. Criterion was reached when the proportion of correct choices was greater than or equal to 0.8 (marked by horizontal dotted line) on two consecutive sessions. Note that individuals tended to perform around chance levels or better at the beginning the initial learning phase; individuals began showing signs of improvement even within the first session. In contrast, individuals performed poorly during the first two reversal sessions as individuals tended to persist on the initially rewarded cup.

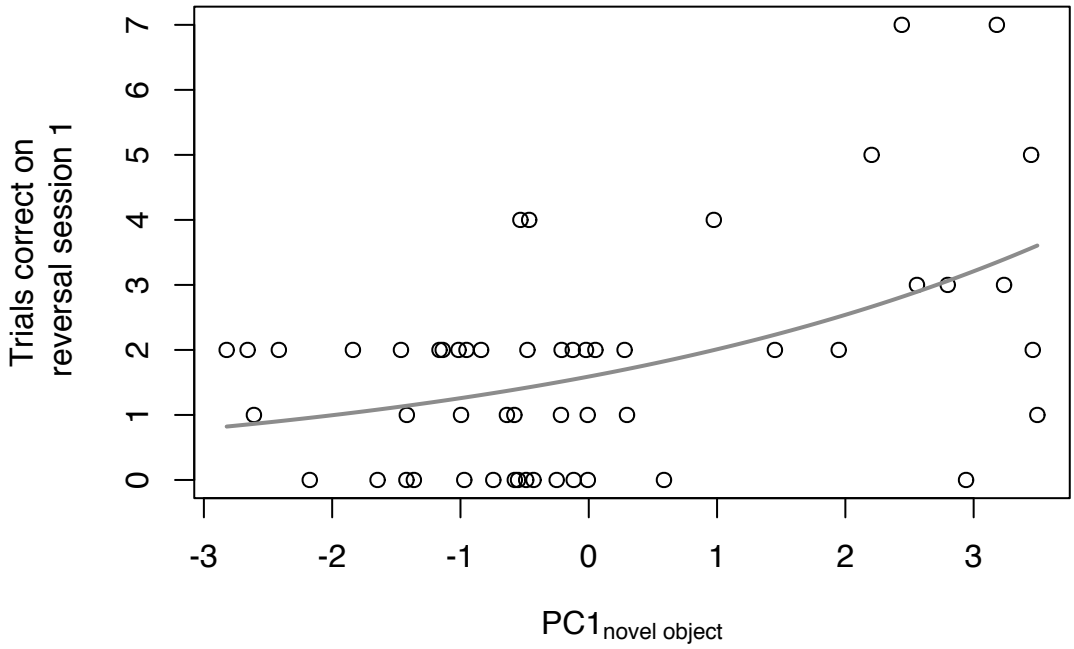


Figure 3.3. Individual differences in behavioral reaction to a novel object were correlated with performance during the first reversal session. Fish that took longer to approach and spend time near and oriented to a novel object (higher $PC1_{novelobject}$) chose the rewarded cup more frequently during the first reversal session.

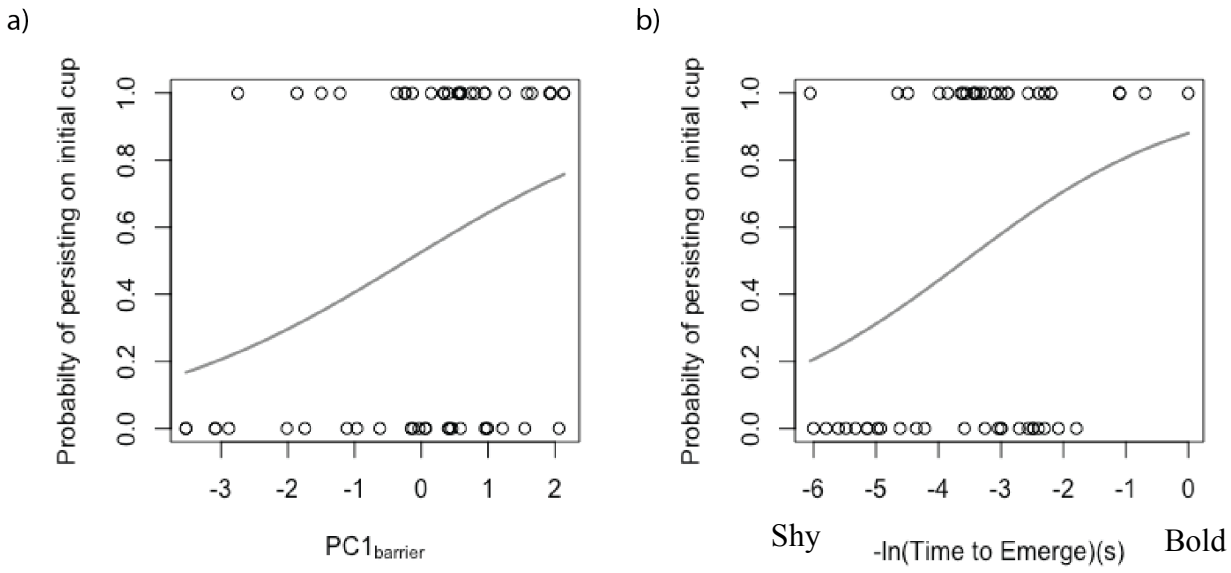


Figure 3.4. a) Behavior during the barrier detour task and b) boldness were associated with persistence on the initially rewarded cup during the first reversal trial. Fish that spent more time at the apex of the barrier ($PC1_{barrier}$, a) and that were faster to emerge from a shelter into a novel environment (time to emerge, b) were more likely to revisit the cup that had been rewarded during initial learning.

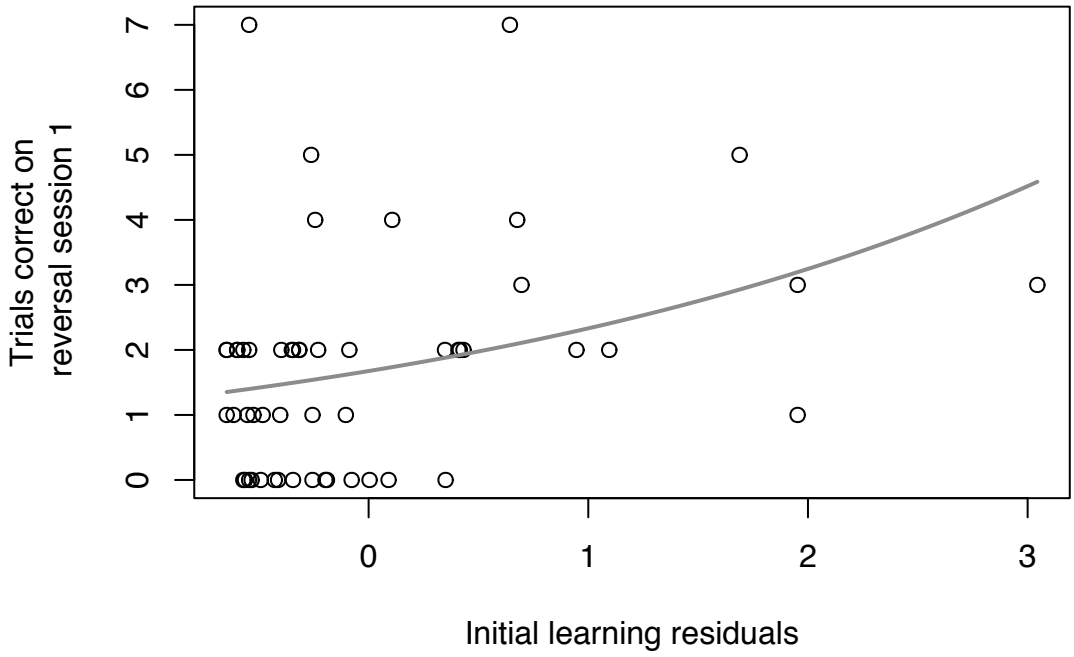


Figure 3.5. Trade-off between initial and reversal learning. Fish that took longer to reach criterion during the initial learning phase performed better on the first ten reversal trials. Initial learning residuals are controlled for body length.

REFERENCES

Amiel, J. J., Lindström, T., & Shine, R. (2014). Egg incubation effects generate positive correlations between size, speed and learning ability in young lizards. *Animal cognition*, *17*(2), 337-347.

Anderson, M. C., & Neely, J. H. (1996). Interference and inhibition in memory retrieval. In *Memory* (pp. 237-313). Academic Press.

Aron, E. N., & Aron, A. (1997). Sensory-processing sensitivity and its relation to introversion and emotionality. *Journal of personality and social psychology*, *73*(2), 345.

Aron, E. N., Aron, A., & Jagiellowicz, J. (2012). Sensory processing sensitivity: A review in the light of the evolution of biological responsivity. *Personality and Social Psychology Review*, *16*(3), 262-282.

Audet, J. N., & Lefebvre, L. (2017). What's flexible in behavioral flexibility? *Behavioral Ecology*, *28*(4), 943-947.

Bebus, S. E., Small, T. W., Jones, B. C., Elderbrock, E. K., & Schoech, S. J. (2016). Associative learning is inversely related to reversal learning and varies with nestling corticosterone exposure. *Animal Behaviour*, *111*, 251-260.

Bensky, M. K., Paitz, R., Pereira, L., & Bell, A. M. (2017). Testing the predictions of coping styles theory in threespined sticklebacks. *Behavioural processes*, *136*, 1-10.

Bensky, M. K., & Bell, A. M. (2018). Intraspecific variation in cue-specific learning in sticklebacks. *Animal Behaviour*, *137*, 161-168.

Benus, R. F., Bohus, B., Koolhaas, J. M., & Van Oortmerssen, G. A. (1990). Behavioural strategies of aggressive and non-aggressive male mice in response to inescapable shock. *Behavioural Processes*, *21*(2-3), 127-141.

Bolhuis, J. E., Schouten, W. G., de Leeuw, J. A., Schrama, J. W., & Wiegant, V. M. (2004). Individual coping characteristics, rearing conditions and behavioural flexibility in pigs. *Behavioural Brain Research*, *152*(2), 351-360.

Boogert, N. J., Madden, J. R., Morand-Ferron, J., & Thornton, A. (2018). Measuring and understanding individual differences in cognition.

Boulougouris, V., Glennon, J. C., & Robbins, T. W. (2008). Dissociable effects of selective 5-HT 2A and 5-HT 2C receptor antagonists on serial spatial reversal learning in rats. *Neuropsychopharmacology*, *33*(8), 2007.

Brown, C., & Braithwaite, V. A. (2004). Size matters: a test of boldness in eight populations of the poeciliid Brachyraphis episcopi. *Animal Behaviour*, *68*(6), 1325-1329.

Carere, C., & Locurto, C. (2011). Interaction between animal personality and animal cognition. *Current Zoology*, *57*(4), 491-498.

Cauchoix, M., Chow, P. K. Y., van Horik, J. O., Atance, C. M., Barbeau, E. J., Barragan-Jason, G., ... & Cauchard, L. (2018). The repeatability of cognitive performance: a meta-analysis. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *373*(1756), 20170281.

Chamberlain, S. R., Müller, U., Blackwell, A. D., Clark, L., Robbins, T. W., & Sahakian, B. J. (2006). Neurochemical modulation of response inhibition and probabilistic learning in humans. *Science*, *311*(5762), 861-863.

Chow, P. K. Y., Leaver, L. A., Wang, M., & Lea, S. E. (2017). Touch screen assays of behavioural flexibility and error characteristics in Eastern grey squirrels (Sciurus carolinensis). *Animal cognition*, *20*(3), 459-471.

Cools, R., Frank, M. J., Gibbs, S. E., Miyakawa, A., Jagust, W., & D'Esposito, M. (2009). Striatal dopamine predicts outcome-specific reversal learning and its sensitivity to dopaminergic drug administration. *Journal of Neuroscience*, *29*(5), 1538-1543.

Croston, R., Branch, C. L., Pitera, A. M., Kozlovsky, D. Y., Bridge, E. S., Parchman, T. L., & Pravosudov, V. V. (2017). Predictably harsh environment is associated with reduced cognitive flexibility in wild food-caching mountain chickadees. *Animal behaviour*, *123*, 139-149.

De Lourdes Ruiz-Gomez, M., Huntingford, F. A., Øverli, Ø., Thörnqvist, P. O., & Höglund, E. (2011). Response to environmental change in rainbow trout selected for divergent stress coping styles. *Physiology & behavior*, *102*(3-4), 317-322.

Den Ouden, H. E., Daw, N. D., Fernandez, G., Elshout, J. A., Rijpkema, M., Hoogman, M., ... & Cools, R. (2013). Dissociable effects of dopamine and serotonin on reversal learning. *Neuron*, *80*(4), 1090-1100.

Dill, L. M. (1983). Adaptive flexibility in the foraging behavior of fishes. *Canadian Journal of Fisheries and Aquatic Sciences*, *40*(4), 398-408.

Dougherty, L. R., & Guillette, L. M. (2018). Linking personality and cognition: a meta-analysis. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *373*(1756), 20170282.

Foster, S. A., & Sih, A. (2013). Behavioural plasticity and evolution. *Animal Behaviour*, *5*(85), 1003.

Ghalambor, C. K., McKay, J. K., Carroll, S. P., & Reznick, D. N. (2007). Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Functional ecology*, *21*(3), 394-407.

Griffin, A. S., Guillette, L. M., & Healy, S. D. (2015). Cognition and personality: an analysis of an emerging field. *Trends in ecology & evolution*, *30*(4), 207-214.

Hadfield, J. D. (2010). MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R package. *Journal of Statistical Software*, *33*(2), 1-22.

Hampton, R. R., & Shettleworth, S. J. (1998). Proactive interference, recency, and associative strength: Comparisons of black-capped chickadees and dark-eyed juncos. *Animal Learning & Behavior*, *26*(4), 475-485.

Izquierdo, A., Brigman, J. L., Radke, A. K., Rudebeck, P. H., & Holmes, A. (2017). The neural basis of reversal learning: an updated perspective. *Neuroscience*, *345*, 12-26.

Izquierdo, A., & Jentsch, J. D. (2012). Reversal learning as a measure of impulsive and compulsive behavior in addictions. *Psychopharmacology*, *219*(2), 607-620.

Lewis, J. L., & Kamil, A. C. (2006). Interference effects in the memory for serially presented locations in clark's nutcrackers, Nucifraga columbiana. *Journal of Experimental Psychology: Animal Behavior Processes*, *32*(4), 407.

Lima, S. L. (2009). Predators and the breeding bird: behavioral and reproductive flexibility under the risk of predation. *Biological reviews*, *84*(3), 485-513.

R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Roberts, A. C., & Wallis, J. D. (2000). Inhibitory control and affective processing in the prefrontal cortex: neuropsychological studies in the common marmoset. *Cerebral Cortex*, *10*(3), 252-262.

Rystrom, T. L., Bakker, T., & Rick, I. P. (2019). Mate assessment behavior is correlated to learning ability in female threespine sticklebacks. *Current Zoology*.

Schoenbaum, G., Roesch, M. R., Stalnaker, T. A., & Takahashi, Y. K. (2009). A new perspective on the role of the orbitofrontal cortex in adaptive behaviour. *Nature Reviews Neuroscience*, *10*(12), 885.

Shine, R., O'Donnell, R. P., Langkilde, T., Wall, M. D., & Mason, R. T. (2005). Snakes in search of sex: the relation between mate-locating ability and mating success in male garter snakes. *Animal Behaviour*, *69*(6), 1251-1258.

Shine, R., Webb, J. K., Lane, A., & Mason, R. T. (2005). Mate location tactics in garter snakes: effects of rival males, interrupted trails and non-pheromonal cues. *Functional Ecology*, *19*(6), 1017-1024.

Sih, A., & Del Giudice, M. (2012). Linking behavioural syndromes and cognition: a behavioural ecology perspective. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *367*(1603), 2762-2772.

Stamps, J. A. (2016). Individual differences in behavioural plasticities. *Biological Reviews*, *91*(2), 534-567.

Tello-Ramos, M. C., Branch, C. L., Kozlovsky, D. Y., Pitera, A. M., & Pravosudov, V. V. (2019). Spatial memory and cognitive flexibility trade-offs: To be or not to be flexible, that is the question. *Animal Behaviour*, *147*, 129-136.

Völter, C. J., Tinklenberg, B., Call, J., & Seed, A. M. (2018). Comparative psychometrics: establishing what differs is central to understanding what evolves. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *373*(1756), 20170283.

West-Eberhard, M. J. (2003). *Developmental plasticity and evolution*. Oxford University Press.

Wolf, M., Van Doorn, G. S., & Weissing, F. J. (2008). Evolutionary emergence of responsive and unresponsive personalities. *Proceedings of the National Academy of Sciences*, *105*(41), 15825-15830.

CHAPTER 4: THE EVOLUTION OF BEHAVIORAL AND COGNITIVE TRAITS OVER THE COURSE OF THE INVASION OF FRESHWATER BY MARINE STICKLEBACKS (*GASTEROSTEUS ACULEATUS*)

ABSTRACT

Species invasions provide an opportunity to study how traits evolve as organisms move into novel environments. The invasion process might act as a filter either via nonrandom dispersal and/or selection, thereby leading to phenotypic differences between populations at the front of the invasion and the source population. Several authors have suggested that behavior and cognition might facilitate the invasion process, but there are few empirical tests. Here, we test the hypothesis that increased neophilia and inhibitory control are beneficial for colonizing new environments, and thus evolve over the course of a biological invasion. This was done by measuring natural variation in these behaviors among genetically-differentiated populations of threespined stickleback that were reared in a common garden. We compared sticklebacks from two populations from the ancestral marine environment to sticklebacks from four freshwater lakes that differ in time since establishment. We predicted that derived freshwater populations would be more neophilic and exhibit higher levels of inhibitory control (i.e. more likely to abandon a previously-established behavior pattern) compared to sticklebacks from the ancestral source population. There was substantial variation in both traits among populations, and suggestive evidence that derived populations were more neophilic and had higher inhibitory control than marine fish. Differences between very recently derived freshwater populations suggest that these differences can undergo selection relatively rapidly, though nonrandom

dispersal cannot be excluded as a potential explanation. These findings are consistent with the hypothesis that cognitive traits have played an important role in allowing stickleback to successfully colonize freshwater habitats, and that these traits have evolved through the invasion process.

INTRODUCTION

Understanding the factors that allow a species to expand its range, and/or adapt to changing habitats, is increasingly important as concerns of climate change and anthropogenic effects continue to grow. Studying biological invasions, or populations that are the result of successful habitat establishment, provide a great opportunity to identify the factors that allow certain species or populations to excel in novel environments (Williamson and Fitter 1996). Invasion influences the evolutionary trajectories of both the invading and native species (Mooney and Cleland 2001). In addition to the importance of propagule pressure (Lockwood et al., 2005; 2009), stochasticity, and the opening of new niches at the edge of species boundaries, there is growing evidence that particular traits might promote invasion. For example, r-selected life histories (Capellini et al. 2015) and habitat breadth (Blackburn et al. 2009) could promote the expansion of a species' range, though findings tend to vary across species/taxa (Hayes and Barry 2008; Kolar and Lodge 2001). Here, we look to test the hypothesis that behavior and cognition may also be important traits that can help explain success in colonizing novel environments.

The study of biological invasions has benefitted from viewing invasion as a multi-stage process requiring a population to be introduced to a new environment, to become established and to ultimately spread in order to be successful (Blackburn *et al.* 2011; Chapple *et al.* 2012;

Lockwood *et al.* 2013). Different traits might be important at different stages of invasion (Blackburn 2011; Chapple and Wong 2016) and the different stages of invasion might act as a filter either via nonrandom dispersal and/or selection, ultimately leading to phenotypic differences between the population at the front of the invasion and its source population (Sakai et al. 2001; Suarez and Tsutsui 2008). Therefore comparing populations of the same species at different stages of the invasion process can provide insights into how and why organisms change as they move into new environments (Castro et al. 2016; Whitney and Gabber 2008). In particular, rearing organisms from different populations that are at different stages of the invasion process in a common garden can reveal whether traits that vary among these populations reflect phenotypic plasticity in response to variation in environmental conditions or evolved differences (e.g., Castro et al. 2016); differences among populations that are preserved in a common garden are likely to reflect evolved, genetically based differences.

Behavioral and cognitive traits are increasingly recognized as potentially important for mediating the ability of animals to move into and become established in new environments (Chapple et al. 2012; Holway and Suarez 1999; Sih et al. 2010; Wright et al. 2010). Behavioral plasticity can help animals excel in new environments by allowing them to rapidly adjust their phenotype in response to changes in environmental cues (Coppens et al. 2010; Wolf et al. 2008). Recent theory has pointed out that cognitive processes -- how animals perceive, process and retain information about their environment and then use that information to make decisions (Shettleworth 2010) – are likely to be especially important in mediating the ability of animals to enter new habitats, to exploit new niches, to become established and to spread (Griffin et al. 2016; Wright et al. 2010). For example, colonists have to be willing to approach and interact with novel stimuli in order to gain information about their new environment, and therefore they

might be expected to exhibit high levels of novelty-seeking behavior (i.e., neophilia; Mettke-Hoffmann 2009). Additionally, previously successful behavioral patterns may no longer be successful in new environments, and so invading individuals need to be able to stop persisting on ineffective responses and flexible enough to attempt new approaches (Griffin et al. 2016), therefore invaders might be expected to exhibit greater inhibitory control.

Threespined sticklebacks (*Gasterosteus aculeatus*) are a good model system for studying behavioral and cognitive traits associated with the invasion process because they are expert colonists: marine sticklebacks are renowned for being able to move into new freshwater environments, and rapidly adapt to them and diversify (Bell and Foster 1994). Sticklebacks in freshwater lakes in Alaska offer an opportunity to examine trait evolution over the course of an invasion because there are some freshwater lakes where sticklebacks have occurred since the last glaciation and other freshwater lakes that have only recently been colonized by marine sticklebacks (Bell et al. 2004; Bell et al. 2016). Moreover, sticklebacks are renowned for their intraspecific variation in behavior (e.g., Bell 2005; Huntingford 1976) and cognition (e.g., Bensky and Bell 2018; Girvan & Braithwaite 1998; Odling-Smee et al. 2003), and are therefore suitable subjects for studying how behavioral and cognitive traits might evolve over the course of invasion.

In this study, we use the stickleback system to test the hypothesis that specific behavioral and cognitive traits improve invasion success (Griffin et al. 2016) by comparing sticklebacks from ancestral anadromous marine populations, two freshwater populations that were recently established (8 and 34 generations before sampling, respectively) and two freshwater populations that were established thousands of years ago. If motivation and willingness to approach and investigate novel stimuli helps animals become established in a new environment, then we

predict that derived freshwater populations will be more neophilic than their marine ancestors. If the ability to quickly inhibit old and ineffective behaviors helps invading animals succeed in a new environment, then we predict that freshwater populations will show high levels of inhibitory control (i.e. persist less on ineffective behavioral patterns and quickly try alternative behaviors), while their marine ancestors will show low inhibitory control (i.e., routinized persistence on old behaviors even when they are no longer reinforced).

Several authors have suggested that studying cognition and behavior during the very early stages of invasion is likely to be especially insightful (Chapple et al. 2012; Chapple and Wong. 2016; Wright et al. 2010) because traits related to behavioral flexibility might be strongly favored at the leading edge of the invasion front (Wright et al. 2010). If this is the case, we might expect sticklebacks from newly derived populations to be especially neophilic and exhibit high levels of inhibitory control relative to sticklebacks from both well-established freshwater populations and from marine populations. Alternatively, neophilia and inhibitory control might be consistently favored throughout the entire invasion process, in which case the newly derived freshwater populations might be either similar to the well-established freshwater populations or intermediate between marine and well-established populations. Because the two newly derived populations differ in time since establishment, we could examine changes that might be occurring over very short (tens of generations) time scales.

By recording the behavior of full sibs from multiple families from multiple populations that had been reared under common garden conditions in the lab, we used these data to assess whether the differences in cognitive and behavioral traits are likely to reflect heritable variation. Importantly, we elected to rear and measure behavior in freshwater (~5ppt), thereby simulating the conditions that marine sticklebacks encounter when they move into freshwater. Because the

marine populations studied here are naturally anadromous (Rabbit Slough: Bell et al. 2016; Resurrection Bay: Personal communication with Rich King), i.e. they spawn in fresh/brackish waters, their early offspring development typically occurs under relatively low salinity conditions.

METHODS

Overview of the experiment

The goal of this study was to test the hypothesis that behavioral and cognitive traits evolve when marine sticklebacks invade freshwater environments. To that end, we reared the offspring of stickleback from multiple marine and freshwater populations of sticklebacks under "common garden" conditions in the lab, and measured their response to a novel object (meant to measure neophilia) and performance on a barrier detour task (meant to measure inhibitory control) as subadults.

Collecting stickleback

Adult stickleback were collected via minnow traps from six populations ranging from the Matanuska-Susitna Valley to the Kenai Peninsula of Alaska (Table 4.1; Figure 4.1) during June 2017. Two populations (Rabbit Slough and Resurrection Bay) occur in the ancestral marine environment, while the remaining four populations occur in freshwater. Two of the freshwater populations (Big Beaver and Cornelius, hereafter referred to as "well established") are derived from natural colonization events that presumably occurred hundreds to thousands of years ago, after the last glacial maximum, while the other two freshwater populations are "newly-

established" via recolonization (Loberg: 28-34 years prior to collection; Bell et al. 2004) or experimental seeding (Cheney: 8 years prior to collection; Bell et al. 2016). As is typical for sticklebacks, the marine populations are only weakly genetically differentiated from each other (F_{ST} = 0.0076; Hohenlohe et al. 2010) while the freshwater populations are more strongly genetically differentiated (Mike Bell, Krishna Veeramah, personal communication).

Eggs were fertilized in the field following previously established protocols (see Wund et al. 2012; 2015). Briefly, males from each population were euthanized using an overdose of buffered tricaine methanesolfonate (MS-222), and their testes were immediately dissected and macerated. Eggs were then gently extruded from gravid females from the same population into a petri dish and the macerated testes were pipetted over the eggs to fertilize them. Next, distilled water with 6 ppt Instant Ocean ® was used to repeatedly rinse the newly fertilized clutches before being stored in that solution in the petri dish. The clutches were then inspected daily for proper development; dead embryos and unfertilized eggs were removed, and the water was changed. Two to three days post fertilization, the eggs were transferred to 50 mL canonical tubes filled with the same water solution and shipped overnight in coolers filled with ice packs to the University of Illinois Urbana-Champaign.

Rearing fish in the lab

Upon arrival, each clutch was moved to its own tank (9.5L 32 x 21 x 19 cm) where the embryos were incubated in a cup with a mesh bottom and placed over an air bubbler. Each tank was lined with gravel and had a refuge (plastic "plant"). Embryos were checked daily and dead embryos were removed. Upon hatching (8-13 days post fertilization) fry were fed brine shrimp daily. Once the eggs had hatched the mesh cup and bubbler were removed. Fish were kept at

60°F with an even light cycle (12L:12D) for the entirety of the experiment. All families were kept on one of two recirculating flow through water racks, which consisted of a series of particular, biological, and IV filters and had three different shelves (Aquaneering , San Diego, USA). 10% of each tank's water was replaced each day. Family tank position was pseudo-randomly assigned so that all populations were evenly distributed across both racks and the three levels of shelves.

At approximately two months of age, the fish were gradually introduced to a mixed diet of frozen bloodworms, frozen brine shrimp, and frozen Mysis shrimp and were fed ad lib once a day. To prevent overcrowding, families were culled to a maximum of 30 fish per tank at two months, and to a maximum of 15 fish at approximately six months.

Behavioral testing

18 observation tanks (36 cm long x 33 cm wide x 24 cm high (26.5 L)) were used for blocks of behavioral testing. When the fish were approximately eight months of age (average standard length of first block 40.66 +/- 0.93 SE; n = 18), the testing phase of the experiment began. Families, sexes and populations were measured in a pseudorandomized order, such that male and female offspring from different families and from different populations were measured in the same block. Individuals were randomly selected from each family, and their weight and length were measured. Then, their sides were then swabbed with a sterile cotton swab in order to non-invasively determine the sex of each fish. Total genomic DNA was extracted using the DNEasy® Blood and Tissue Kit (Qiagen, Venlo, Netherlands), and sex was determined using a genetic marker, per the methods of Peichel et al. (2004). The observation tanks were plexiglass, and lines were drawn on the outside bottom of each tank in order to separate it into equal thirds

(i.e., left, center, right). Each observation tank was then lined with gravel, but the floor was cleared immediately around the lines so that they were still visible from a "top-down" view. A plastic "plant" was placed into the middle third of each tank to provide a refuge. Opaque dividers were inserted between the observation tanks during behavioral testing; otherwise individuals had visual access to fish in neighboring tanks. During the testing phase of the experiment, fish were only fed during the behavioral tests to help maintain motivation.

Acclimation Phase

In order to ensure that an individual had acclimated to the observation tank and was motivated to eat during the behavioral tests, the individual was presented with food, and the individual had to eat the food within 10 minutes on three consecutive days in order to proceed to the next step. On average, it took 5.1 days for fish to meet this criterion (range = 3 to 17 days). More details about the acclimation procedure are in Chapter 3.

Novel Object Test

The purpose of this test was to characterize variation in neophilia within and among the populations. To that end, individuals' response to a novel object (toy lion; 10L x 7H cm; TERRA by Battat, Montreal, Canada) was recorded the day after the fish met criterion in the acclimation phase. The toy lion was selected as a novel object because the fish had no prior experience with this object, there was no presumed evolutionary history with the object's shape, and it was made up of neutral colors.

To set up the trial, the plastic plant was removed, plastic dividers were placed on all sides of the tank, and a mirror was positioned at a 45-degree angle above the tank to provide a top-

down view. A camcorder (JVC Everio HD Hard Dish Camcorder Model No: GZ-HD40U) was used to record the trial via the mirror. A perforated tank divider was used to block the fish from accessing the back part of the tank that was out of the camera's view. One of the outside thirds of the tank was randomly selected, and a circular blind was used to corral the individual into that area. The novel object was then placed in the opposite end and the individual was then left undisturbed for five minutes.

After five minutes, the blind was removed. The observer then recorded the individual's behavior for five minutes after their first approach of the novel object (i.e., first time within one body length of the novel object and oriented directly towards it). Specifically, the observer recorded how much time was spent orienting towards the novel object while the individual was in the same third of the tank as the object. We interpret more time spent near and oriented towards the novel object as greater time investigating the object (i.e., higher neophilia). Upon completion of the test, the novel object and divider were removed, the plant was returned to the tank, and the blinders and mirror were removed.

Barrier Detour Task

The purpose of this task was to characterize variation within and among populations in inhibitory control as measured on a barrier detour task (Kabadayi et al. 2018), following methods similar to those described in Chapter 3. Training for the barrier detour task started on the same day the novel object test was completed. All barrier detour task-related trials occurred in a separate testing tank. This tank had the same dimensions as the individual's home observation tank. No gravel was present to help increase the salience of a food reward that would be placed in the middle of the tank for each trial. The goals of this training were to train the fish that there

would consistently be a food reward in the middle of the tank, establish food motivation in this context, and create a prepotent response of leaving a shelter to directly approach and eat the food reward.

Individuals were trained for one session per day, with each session comprising four trials. To begin the session, the individual was gently scooped with a white cup from their home tank and transferred to an opaque shelter that was then placed into the back-center of the testing tank and was left undisturbed for three minutes. A 60W mm circle was initially drawn on the center of the floor of the test tank (~7 cm from the entrance of the shelter), and a single blood worm was placed within that circle. To start the trial, the observer removed a cork from the side of the shelter and the fish was given ten minutes to exit. Upon exiting the fish was allowed five minutes to eat the worm.

After eating the worm, the fish was placed back into the shelter in preparation for the next trial. If the fish did not emerge from the shelter within ten minutes after the cork was pulled or eat within five minutes after emergence, the observer recorded the maximum times for these behaviors, removed the food reward and gently poured the fish out of the shelter if necessary. Between trials fish were allowed to reacclimate to the shelter for two minutes. After the fourth trial of the day the fish were returned to their home tanks.

Training for the barrier task was criterion based. In order to move on to the barrier task, the individual had to emerge from the shelter within 10 minutes and directly approach and eat the food reward within five seconds on three out of the four trials. The one failed attempt could not be on the fourth trial; this requirement was to ensure that the fish would be motivated throughout the four trials. Fish were given a maximum of four days to reach criterion. If an individual did not reach criterion for the barrier task within four days, an additional individual from that family

was sampled later in the experiment if possible in order to maximize the number of individuals tested within each family. 15 individuals did not reach criterion.

Once an individual had met criterion, the individual moved on to the barrier detour task the following day. This task also consisted of four trials, with two minutes between trials. In order to reinforce the direct search pattern, the first two trials were exactly the same as the training trials. On the third trial a transparent semi-circular barrier was placed between the shelter and food reward. The opening into the barrier was positioned directly in front of the entrance to the shelter. After removing the cork the individual was allowed 30 minutes to emerge from the shelter, navigate around the barrier and eat the food reward. Eleven fish did not solve the task and eat within 30 minutes. The observer recorded the duration of the first bout (no break in contract longer than five seconds) at the "apex" of the barrier, which is the point of the barrier closet to the food reward. Fish that continue to follow the prepotent search pattern established during training should spend more time at this portion of the barrier. We interpret greater time spent at the barrier apex as lower inhibitory control/higher persistence, because those individuals took longer to break away from a previously established behavior pattern. In order to confirm that the fish that spent little time at the barrier apex during the third trial were still motivated to eat, the fish's behavior was observed for a fourth time. During the fourth trial, no barrier was present. All fish approached and ate the food on the fourth trial. Upon completion of the fourth trial, the individual was returned to its home tank.

Altogether, a total of n=262 individuals from n=8-11 families/population (n=2-7 full sibs per family) completed the novel object test and a total of n=247 individuals from n=8-11 families/population (n=1-4 full sibs per family) completed the barrier task.

Statistical Analysis

R 3.5.3 (http://www.r-project.org/) was used for statistical analyses. We used linear mixed models (R Core Team 2016; package = "lme4"; function = "lmer") to examine time spent near and oriented towards the novel object (where more time with the novel object was interpreted as higher neophilia) and time at the apex (where greater time at the apex was interpreted as lower inhibitory control, i.e. higher persistence and lower willingness to try alternative behaviors). Both measures were positively-skewed and were therefore log-transformed to improve normality. Model residuals were also visually inspected for deviations from normality.

We started with full models in which population, sex, and the interaction between the two were included as fixed factors. Body length was included as a covariate. FamilyID nested within population was included as a random variable. Sex x Population, and the main effect of Sex were never statistically significant and were therefore removed from subsequent analyses. The statistical significance of the effect of FamilyID was assessed by AIC (Akaike, 1973), i.e. by comparing models with and without the effect of FamilyID (R Core Team 2016; package = "care"; function = "anova") .

When there was a significant effect of population, post-hoc pairwise comparisons (R Core Team 2016; package = "emmeans"; function = "emmeans") were performed. Post-hoc p-values were adjusted using the Tukey method for multiple comparisons.

RESULTS

Variation in behavior toward the novel object

Body size (body length) influenced the way that sticklebacks reacted to the novel object (β = -0.044, t = -2.134, p=0.034, Figure 4.2, Table 4.2) such that larger fish spent less time near and oriented towards the novel object. There was significant population-level variation in behavior toward the novel object (Table 4.2, Figure 4.3), such that visually, it appears that neophilia increased over the course of invasion as was predicted, but post-hoc tests (Tables 4.3 and 4.4) did not show significant pairwise differences between the populations. There was a trend for sticklebacks from Loberg Lake (which was colonized 28-34 generations prior) to spend more time near and oriented to the novel object compared to sticklebacks from both Rabbit Slough (a marine population, x = 0.844, SE = 0.302, t = 2.793, adjusted p = 0.0723) and Cheney Lake (which was colonized 8 generations prior, x = 0.832, SE = 0.295, t = 2.815, adjusted p = 0.0686).

We did not detect significant variation among families within populations in behavior toward the novel object, as FamilyID did not improve model fit (X^2 = 0.026, p = 0.873; AIC_{with} = 907.25; $AIC_{without}$ = 905.27, Figure 4.4).

Variation in time spent at the barrier apex

There was substantial population-level variation in time spent at the barrier apex (Table 4.2, Figure 4.5), with the overall visual pattern matching our prediction and suggesting that inhibitory control increased over the course of invasion. Statistically, according to post-hoc tests (Tables 4.5 and 4.6), sticklebacks from Cheney Lake spent significantly more time persisting at

the barrier apex compared to sticklebacks from both Loberg ($x = 0.823$, SE = 0.277, $t = 2.968$, adjusted $p = 0.0474$), and Big Beaver ($x = 1.043$, SE = 0.282, $t = 3.704$, adjusted $p = 0.0059$). Sticklebacks from Rabbit Slough spent marginally more time at the barrier apex than sticklebacks from Big Beaver ($x = 0.801$, SE = 0.286, $t = 2.801$, adjusted $p = 0.0710$). We did not detect an effect of body length on time spent at the barrier apex ($\beta = -0.0028$, $t = -0.166$, $p=0.868$, Table 4.2).

In addition to the significant population-level variation, there was also significant variation in time spent at the barrier apex among families within populations ($X^2 = 6.5894$, $p = 0.01026$; $AIC_{with} = 722.69$; $AIC_{without} = 727.28$, Figure 4.6).

DISCUSSION

If behavioral and cognitive traits allow dispersing populations to successfully pass through the stages of invasion, then there should be differences in behavioral and cognitive traits between organisms living in their native versus invaded range, either due to differences in selection pressures or non-random dispersal (Griffin et al. 2016). In the current study, we observed significant population-level variation in response to a novel stimulus and in inhibitory control under common garden conditions. We also observed family-level variation in barrier persistence. These findings suggest a heritable component to both neophilia and inhibitory control, and that these traits can evolve.

We hypothesized that neophilia would be higher in freshwater populations of sticklebacks compared to marine (ancestral) populations, because seeking and/or being willing to investigate novel stimuli may help individuals locate new habitats and discover novel resources.

After correcting for multiple comparisons, post-hoc analysis did not identify any significant pairwise differences between populations. However, examining the observed means lends general support to this prediction with marine populations spending less time on average investigating the novel object. Length was also found to be a significant co-variate in the model: larger fish spent less time investigating the novel object. This effect could reflect differences in age (animals were measured over the course of five months). Indeed, novelty-seeking has been found to decrease with age in other systems (Adriani et al. 1998; Stansfield and Kirsten 2005).

We also hypothesized that higher inhibitory control could benefit individuals colonizing new environments by helping them stop ineffective behavioral patterns in order to attempt new ones. We found some support for this hypothesis: marine populations were more persistent on the barrier task (i.e., longer time at the apex). These marine fish took longer to discontinue the direct approach pattern that had been reinforced during training in order to attempt an alternative approach.

We also observed significant variation in inhibitory control among families within populations, which could reflect genetic variation within populations. However, it is important to note that tank effects could potentially contribute to differences among families because full sibs were kept in the same tank (although families within each population were evenly distributed around the fish room in an attempt to control for location effects). Moreover, the shared tank environment does not necessarily lead to similarities, i.e. because individuals diversify within the same environment to become more different from each other (Bergmüller and Taborsky 2010).

Interestingly, sticklebacks from the two "new" freshwater populations behaved very differently from each other in this study. Fish from Cheney Lake, which was seeded only nine years prior to collection, persisted on the barrier significantly longer than fish from Loberg Lake,

which was recolonized 28-34 years prior to this study. There was also a trend of lower neophilia in Cheney compared to Loberg. Therefore Cheney tended to more closely resemble the marine populations while Loberg was more similar to the established freshwater populations. There are at least two possible explanations for this pattern. First, it is possible that behavioral and cognitive traits can evolve very quickly due to selection over the course of invasion of freshwater, causing Loberg to diverge from the ancestral marine form in as little as 30 generations. Previous studies have indeed suggested that morphological traits in sticklebacks can evolve very rapidly, i.e. within decades (Bell et al. 2004; Bell et al. 2016).

Another possibility is that the phenotypic differences between sticklebacks from Loberg and Cheney reflect differences in the way that the two lakes were colonized; Loberg was naturally colonized, while sticklebacks were experimentally introduced to Cheney. It is possible that sticklebacks from Loberg were more neophilic and exhibited higher inhibitory control because there was nonrandom dispersal of particularly novelty seeking, flexible and uninhibited behavioral types of individuals into Loberg, but a random sample of behavioral types were artificially introduced into Cheney. Further studies tracking how behavioral and cognitive traits change over time in the Cheney population (and similar experimental lakes (e.g., Scout Lake)) could help tease these hypotheses apart.

An important consideration is that sticklebacks from marine and freshwater populations in this study not only differ in whether they are at the start or end of the invasion process, but also in whether they are adapted to relatively high or low salinity environments, respectively, and the animals in this experiment were reared and measured in low salinity conditions. Indeed, an alternative explanation for the population differences observed in this study is that they reflect the effects of salinity rather than stages of invasion. Perhaps sticklebacks from freshwater

populations are more neophilic and have greater inhibitory control because low salinity favors those traits via either plasticity or selection; salinity acclimation has been shown to affect behavior in other fish species (Fanta-Feofiloff et al. 1985). Experiments comparing neophilia and inhibitory control in high versus low salinity conditions is a promising direction for future work. Another possibility is that marine populations behaved differently compared to freshwater populations in this study because the low salinity conditions in this experiment were more stressful to them. We think this is unlikely because we did not detect signs of excessive stress in the marine fish compared to the other populations in terms of overall health and viability, and they naturally experience low salinity conditions as part of their anadromous life history (Rabbit Slough: Bell et al. 2016; Resurrection Bay: Personal communication with Rich King).

Species invasions have important consequences for evolution. Identifying the traits that allow animals to successfully move into new habitats is critical for elucidating the mechanism that drive variation between population (Hayes and Barry 2008; Kolar and Lodge 2001). Propagule pressure, or the size of the dispersing population, is a consistent factor in invasion success (Lockwood et al. 2009; Simberloff 2009), and there is growing appreciation that variation in behavior and cognition can help explain differences in propagule pressure as well as improve our understanding of how successful invasive populations are able to successfully navigate different stages of the invasion process (Chapple et al. 2012; Griffin et al. 2016; Holway and Suarez 1999; Wright et al. 2010). Our data suggest that both neophilia and inhibitory control have evolved over the course of freshwater colonization in stickleback. By capitalizing on the natural experiment provided by the stickleback radiation and a common garden experimental design, the current project provides support for emerging theory about how behavioral and cognitive traits can facilitate and evolve over the course of biological invasions.

ACKNOWLEDGEMENTS

We wish to thank M. A. Wund, J. A. Baker, and the members of the Foster Lab for all of their help and guidance with field collections. We are also grateful to the members of the Bell lab for their help throughout this project. Financial support was provided by the National Science Foundation's Integrative Graduate Education and Research Traineeship program and by the University of Illinois. This material is based upon work supported by the National Science Foundation under Grant No. IOS 1121980, by the National Institutes of Health under award number 2R01GM082937-06A1.

COMPLIANCE WITH ETHICAL STANDARDS

The experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Illinois Urbana-Champaign (IACUC protocol #15077). Fish were caught in the field using baited minnow traps under the approval of the State of Alaska's Department of Fish and Wildlife permit to M. Bensky (FRP P-17-019 and FTP 17A-0020). In the lab, the fish were housed in groups before and after the experiment. All experimental procedures were non-invasive. While the fish were undergoing training they were housed individually, but were given visual access to neighboring fish when they were not participating in active trials in order to enhance their welfare. Fish were transferred from their home tank to the training tanks by gently scooping them in a cup to minimize stress.

TABLES AND FIGURES

Population name	Population type	Latitude/Longitude
Resurrection Bay	Marine	59.970 N, 149.381 W
Rabbit Slough	Marine	61.537 N, 149.220 W
Cheney Lake	"New" Freshwater (5 generations; established 2009)[1]	61.2028 N, 149.7583 W
Loberg Lake	"New" Freshwater (30 generations; established 1983-1988)[2]	61.560 N, 149.258 W
Cornelius Lake	"Established" Freshwater	61.628 N, 149.256 W
Big Beaver Lake	"Established" Freshwater	61.581 N, 149.833 W

[1] Bell, M.A., D.C. Heins, M.A. Wund, F.A. von Hippel, R. Massengill, K. Dunker, G.A. Bristow, and W.E. Aguirre. 2016. Reintroduction of threespine stickleback into Cheney and Scout Lakes, Alaska. Evolutionary Ecology Research 17: 157-178.

[2] Bell, M.A., W.E. Aguirre, and N.J. Buck. 2004. Twelve years of contemporary armor evolution in a threespine stickleback population. Evolution 58:814-824.

Table 4.1. Description of the populations. Number of generations at the time of collection is indicated.

	Time near and oriented		Time at the apex	
Factor	*F*(df)	p-value	*F*(df)	p-value
Population	**3.076(5,62)**	**0.01528**	**3.597 (5,59)**	**0.007**
Length	**4.552(1,235)**	**0.03392**	0.028 (1,239)	0.868

Table 4.2. Linear mixed models testing for the effect of population and body length on time near and oriented to the novel object and time at the apex.

Population	Estimate	SE	Lower CI	Upper CI
Resurrection Bay (Marine)	2.75	0.258	2.24	3.27
Rabbit Slough (Marine)	2.73	0.217	2.29	3.16
Cheney Lake (New)	2.74	0.208	2.32	3.15
Loberg Lake (New)	3.57	0.210	3.15	3.99
Cornelius Lake (Established)	3.49	0.213	3.07	3.92
Big Beaver Lake (Established)	3.17	0.204	2.76	3.58

Confidence level used: 95%

Table 4.3. Estimated marginal mean time spent near and oriented to novel object in each population.

Contrast	Estimate difference	SE	t-ratio	p-value
Resurrection Bay (M) – Rabbit Slough (M)	0.0286	0.333	0.086	1.0000
Resurrection Bay (M) – Cheney (N)	0.0160	0.326	0.049	1.0000
Resurrection Bay (M) – Loberg (N)	-0.8155	0.334	-2.444	0.1574
Resurrection Bay (M) – Cornelius (E)	-0.7386	0.345	-2.140	0.2799
Resurrection Bay (M) – Big Beaver (E)	-0.4150	0.337	-1.232	0.8192
Rabbit Slough (M) – Cheney (N)	-0.0126	0.299	-0.042	1.0000
Rabbit Slough (M) – Loberg (N)	*-0.8441*	*0.302*	*-2.793*	*0.0723*
Rabbit Slough (M) – Cornelius (E)	-0.7672	0.307	-2.502	0.1391
Rabbit Slough (M) – Big Beaver (E)	-0.4436	0.300	-1.480	0.6782
Cheney (N) – Loberg (N)	*-0.8315*	*0.295*	*-2.815*	*0.0686*
Cheney (N) – Cornelius (E)	-0.7546	0.300	-2.514	0.1358
Cheney (N) – Big Beaver (E)	-0.4310	0.293	-1.470	0.6841
Loberg (N) – Cornelius (E)	0.0770	0.298	0.259	0.9998
Loberg (N) – Big Beaver (E)	0.4005	0.292	1.373	0.7425
Cornelius (E) – Big Beaver (E)	0.3236	0.289	1.119	0.8713

P-value adjustment: Tukey method for comparing a family of 6 estimates

M = Marine; N = New Freshwater; E = Established Freshwater

Table 4.4. Estimated mean differences in time spent near and oriented to the novel object between populations.

Population	Estimate	SE	Lower CI	Upper CI
Resurrection Bay (Marine)	2.18	0.239	1.71	2.66
Rabbit Slough (Marine)	2.46	0.203	2.06	2.87
Cheney Lake (New)	2.70	0.196	2.31	3.09
Loberg Lake (New)	1.88	0.195	1.49	2.27
Cornelius Lake (Established)	2.04	0.199	1.64	2.43
Big Beaver Lake (Established)	1.66	0.199	1.26	2.06

Confidence level used: 95%

Table 4.5. Estimated marginal mean time at the apex for each population.

Contrast	Estimate difference	SE	t-ratio	p-value
Resurrection Bay (M) – Rabbit Slough (M)	-0.279	0.310	-0.900	0.9449
Resurrection Bay (M) – Cheney (N)	-0.520	0.304	1.710	0.5306
Resurrection Bay (M) – Loberg (N)	0.303	0.310	0.976	0.9238
Resurrection Bay (M) – Cornelius (E)	0.147	0.320	0.459	0.9973
Resurrection Bay (M) – Big Beaver (E)	0.523	0.317	1.647	0.5709
Rabbit Slough (M) – Cheney (N)	-0.242	0.281	-0.860	0.9544
Rabbit Slough (M) – Loberg (N)	0.582	0.282	2.060	0.3222
Rabbit Slough (M) – Cornelius (E)	0.425	0.287	1.483	0.6759
Rabbit Slough (M) – Big Beaver (E)	0.801	0.286	2.801	0.0710
Cheney (N) – Loberg (N)	**0.823**	**0.277**	**2.968**	**0.0474**
Cheney (N) – Cornelius (E)	0.667	0.282	2.362	0.1860
Cheney (N) – Big Beaver (E)	**1.043**	**0.282**	**3.704**	**0.0059**
Loberg (N) – Cornelius (E)	-0.156	0.277	-0.564	0.9929
Loberg (N) – Big Beaver (E)	0.220	0.278	0.792	0.9678
Cornelius (E) – Big Beaver (E)	0.376	0.276	1.361	0.7495

P-value adjustment: Tukey method for comparing a family of 6 estimates

M = Marine; N = New Freshwater; E = Established Freshwater

Table 4.6. Estimated mean differences in time at the apex between populations

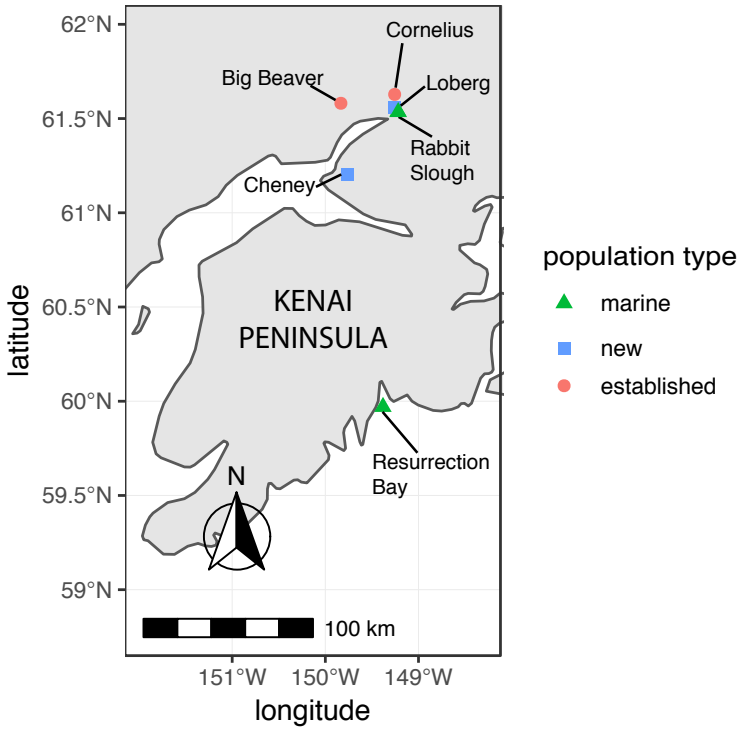


Figure 4.1. Map of sampling sites.

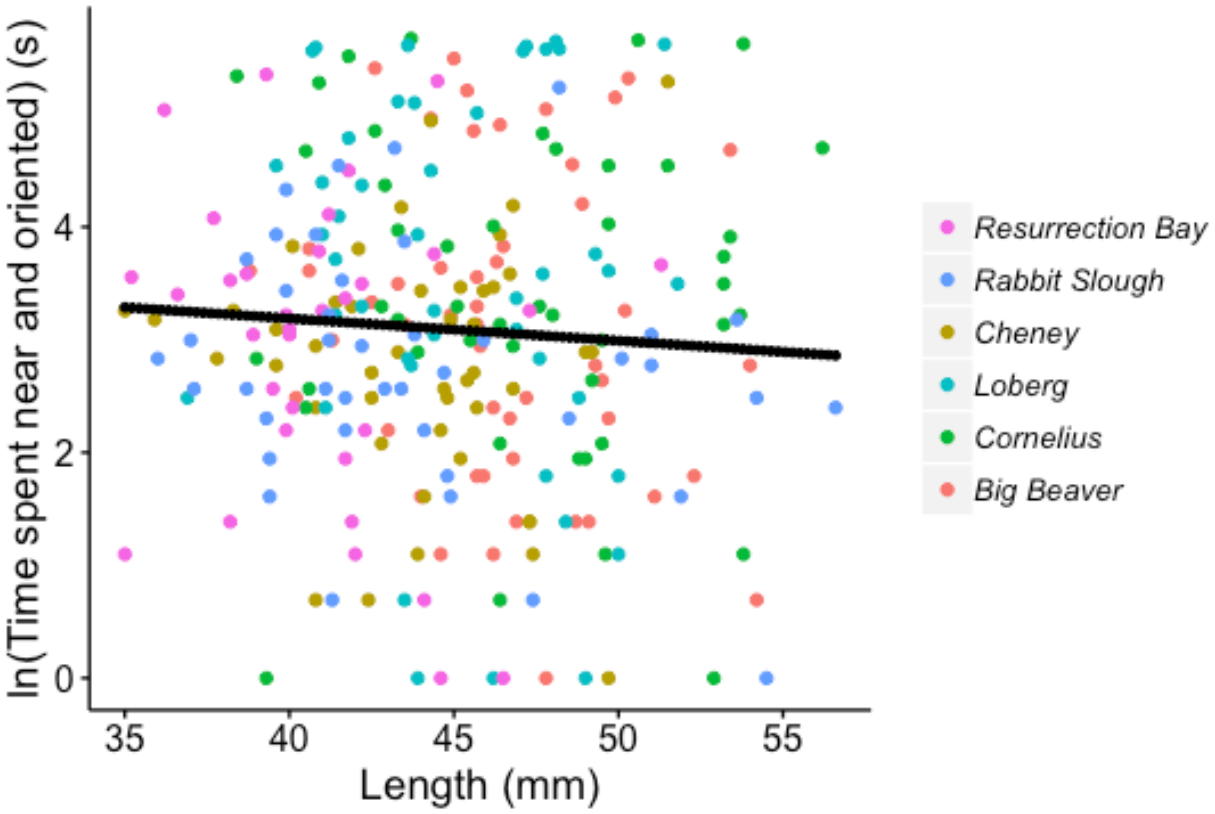


Figure 4.2. Bigger fish spent less time near and oriented to the novel object. Each data point represents a different individual, color coded by population**.**

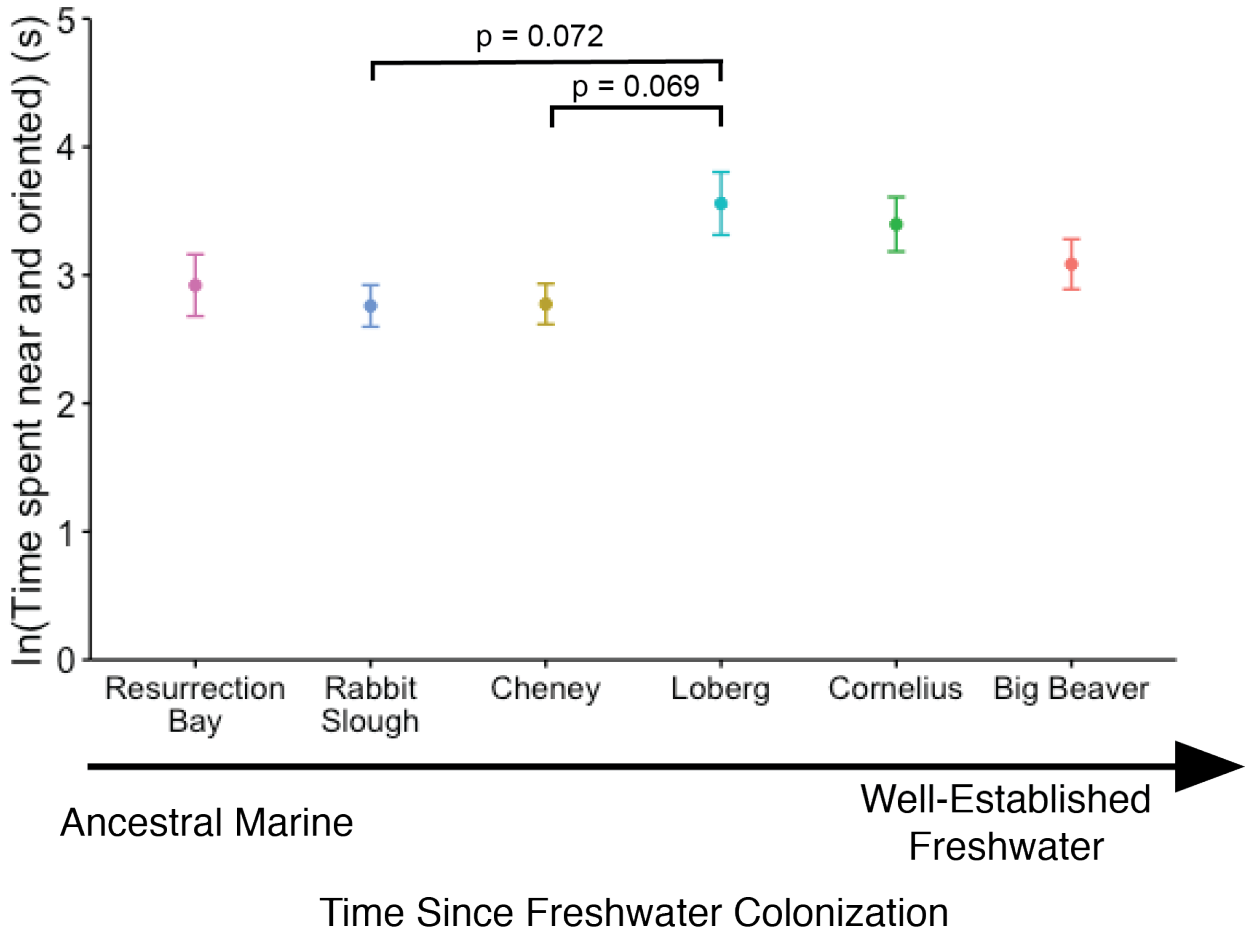


Figure 4.3. Population-level variation in time spent near and oriented to the novel object.

Each data point represents the mean of n=8-11 families with n=2-7 full sibs per family; bars show standard errors.

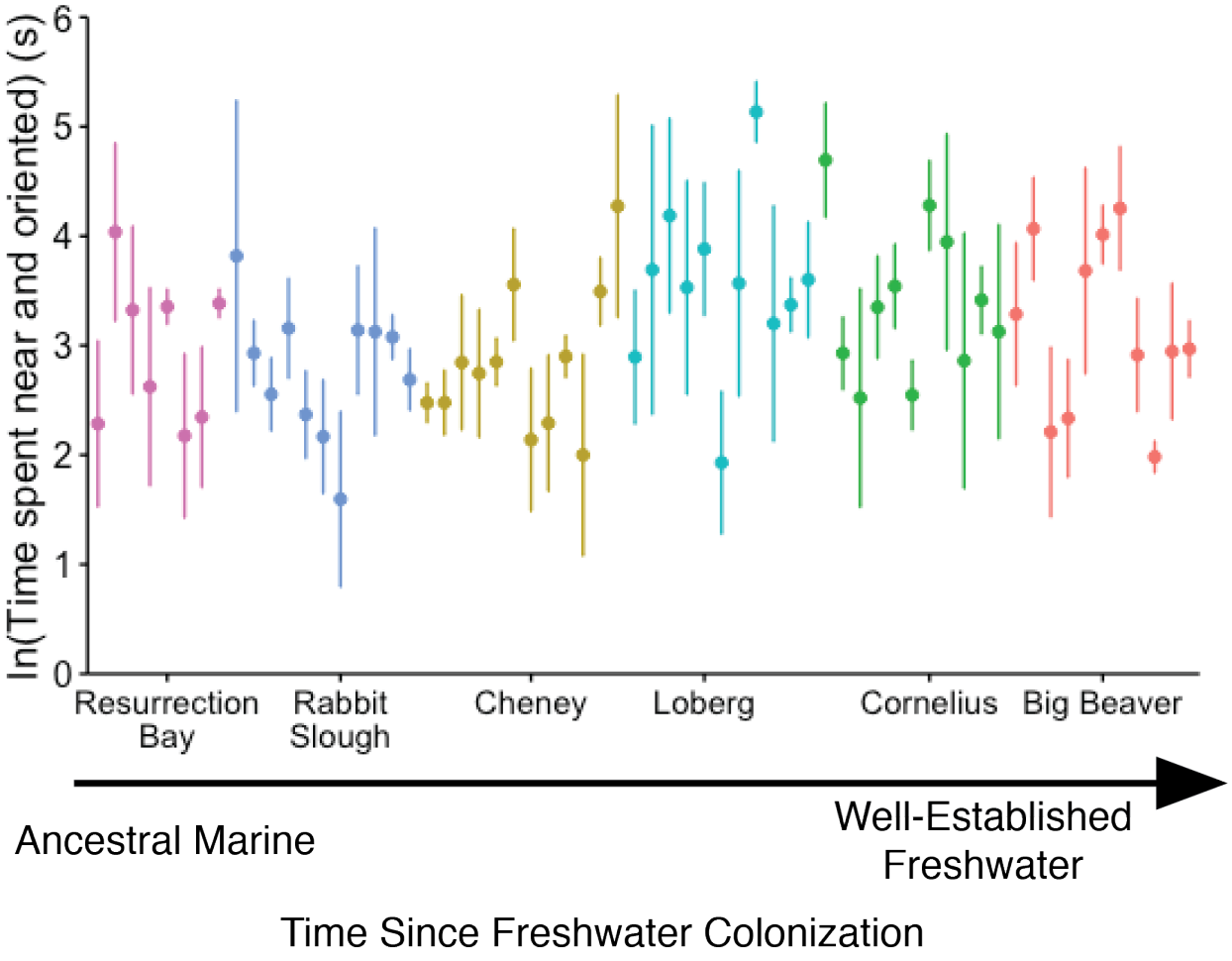


Figure 4.4. Family-level variation in time spent near and oriented to the novel object. Each data point represents the mean (+/- se) of n=2-7 individuals per family, with families color coded by population.

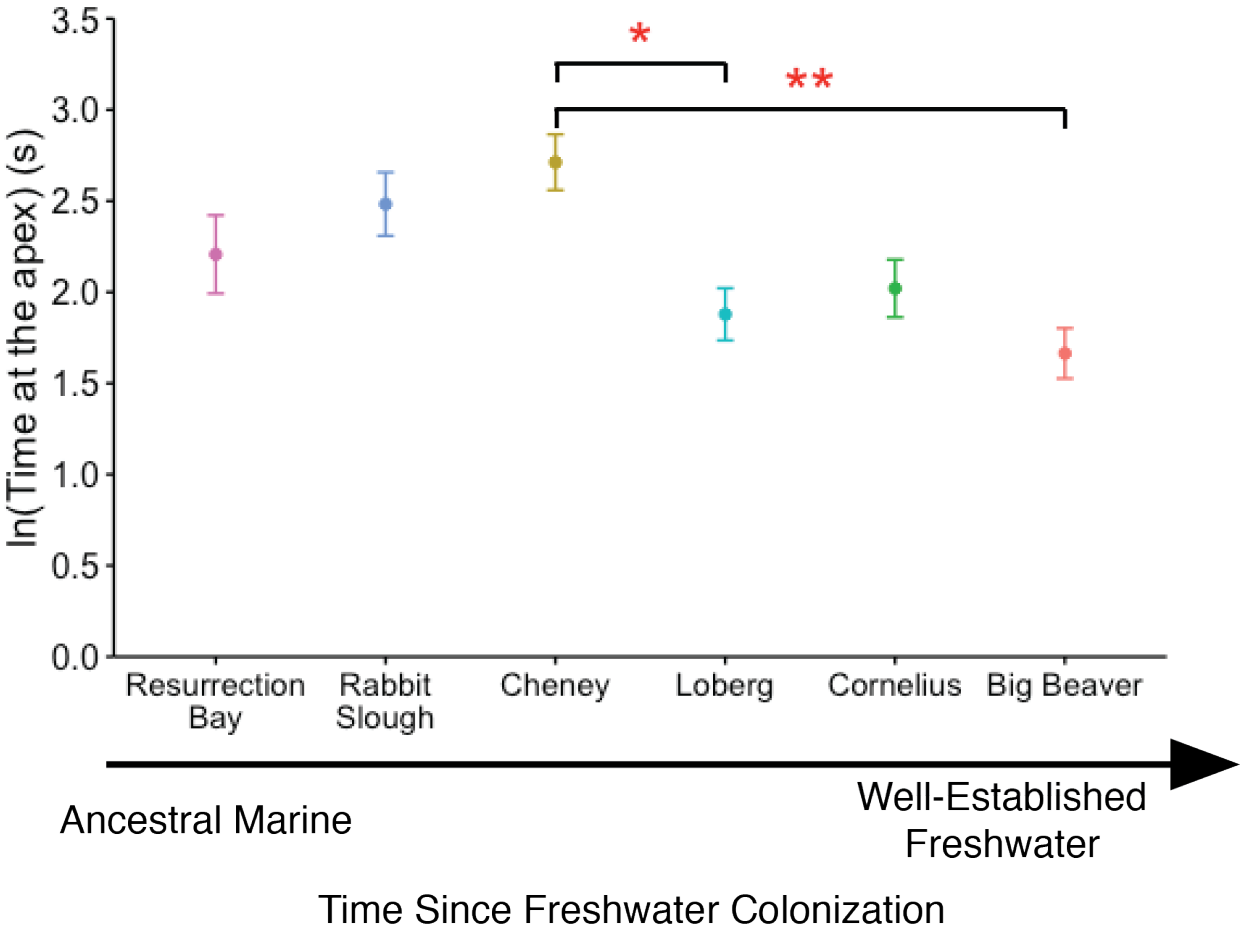


Figure 4.5. Population-level variation in time at the apex. Each data point represents the mean of n=8-11 families with n=1-4 full sibs per family; error bars represent standard error. *P<0.05, **P<0.01.

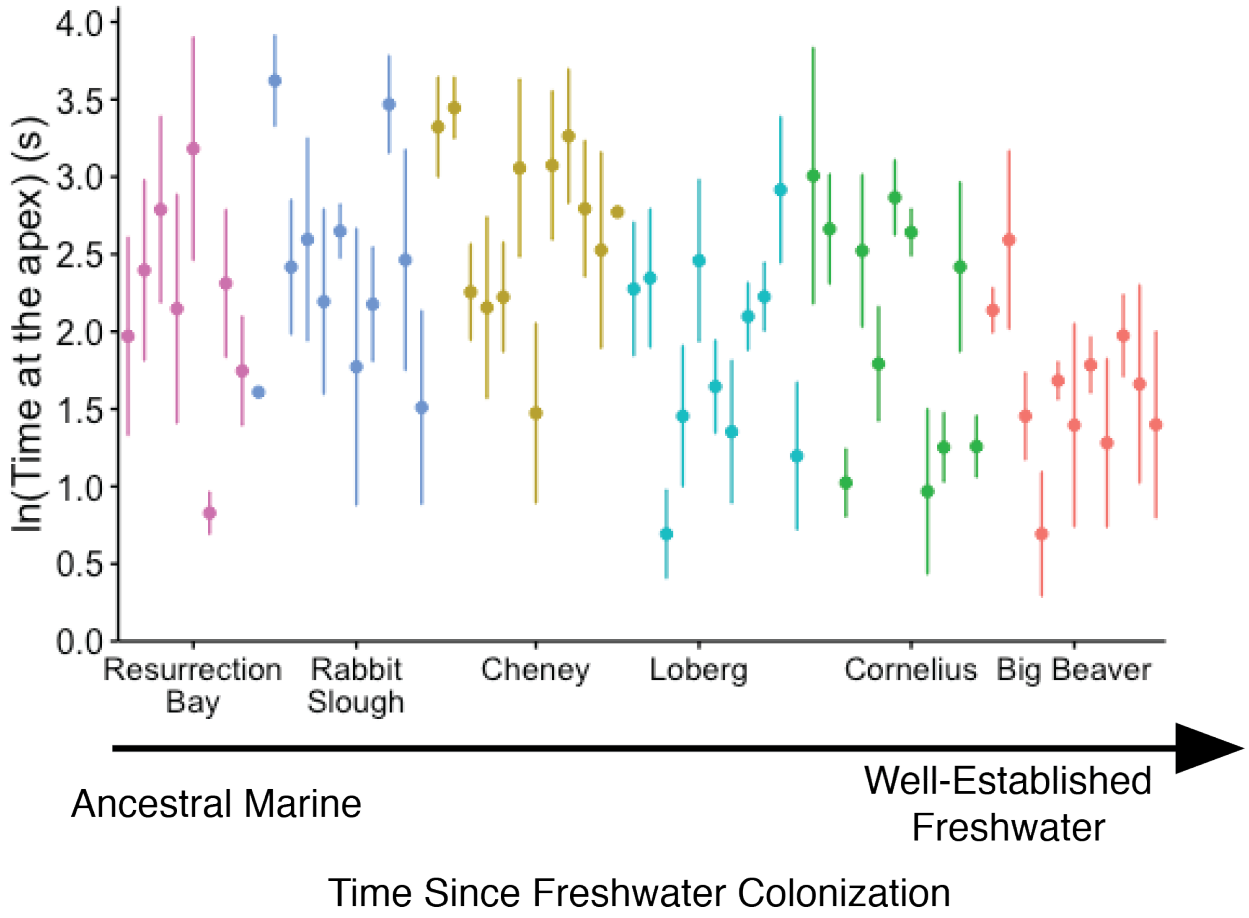


Figure 4.6. Family-level variation in time at the apex. Each data point represents the mean (+/- se) of n=1-4 individuals per family, with families colored coded by population.

REFERENCES

Adriani, W., Chiarotti, F., & Laviola, G. (1998). Elevated novelty seeking and peculiar d-amphetamine sensitization in periadolescent mice compared with adult mice. *Behavioral neuroscience*, *112*(5), 1152.

Akaike, H 1973. Information theory and an extension of the maximum likelihood principle. Pages 267– 281 *in* B. N Petrovand S Caski editors. *Proceedings of the Second International Symposium on Information Theory*. Akademiai Kaido, Budapest, Hungary.

Bell, A. M. (2005). Behavioural differences between individuals and two populations of stickleback (Gasterosteus aculeatus). *Journal of evolutionary biology*, *18*(2), 464-473.

Bell, M. A. (1994). *The evolutionary biology of the threespine stickleback* (pp. 381-398). S. A. Foster (Ed.). Oxford: Oxford University Press.

Bell, M. A., Aguirre, W. E., & Buck, N. J. (2004). Twelve years of contemporary armor evolution in a threespine stickleback population. *Evolution*, *58*(4), 814-824.

Bell, M. A., Heins, D. C., Wund, M. A., von Hippel, F. A., Massengill, R., Dunker, K., ... & Aguirre, W. E. (2016). Reintroduction of threespine stickleback into Cheney and Scout Lakes, Alaska. *Evolutionary Ecology Research*, *17*(2), 157-178.

Bensky, M. K., & Bell, A. M. (2018). Intraspecific variation in cue-specific learning in sticklebacks. *Animal behaviour*, *137*, 161-168.

Bergmüller, R., & Taborsky, M. (2010). Animal personality due to social niche specialisation. *Trends in Ecology & Evolution*, *25*(9), 504-511.

Blackburn, T. M., Cassey, P., & Lockwood, J. L. (2009). The role of species traits in the establishment success of exotic birds. *Global Change Biology*, *15*(12), 2852-2860.

Blackburn, T. M., Pyšek, P., Bacher, S., Carlton, J. T., Duncan, R. P., Jarošík, V., ... & Richardson, D. M. (2011). A proposed unified framework for biological invasions. *Trends in ecology & evolution*, *26*(7), 333-339.

Capellini, I., Baker, J., Allen, W. L., Street, S. E., & Venditti, C. (2015). The role of life history traits in mammalian invasion success. *Ecology Letters*, *18*(10), 1099-1107.

Castro, S., Castro, M., Ferrero, V., Costa, J., Tavares, D., Navarro, L., & Loureiro, J. (2016). Invasion fosters change: independent evolutionary shifts in reproductive traits after Oxalis pes-caprae L. introduction. *Frontiers in plant science*, *7*, 874.

Chapple, D. G., Simmonds, S. M., & Wong, B. B. (2012). Can behavioral and personality traits influence the success of unintentional species introductions? *Trends in ecology & evolution*, *27*(1), 57-64.

Chapple, D. G., & Wong, B. B. (2016). 2 The Role of Behavioural Variation across Different Stages of the Introduction Process. *Biological invasions and animal behaviour*, 7.

Coppens, C. M., de Boer, S. F., & Koolhaas, J. M. (2010). Coping styles and behavioural flexibility: towards underlying mechanisms. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1560), 4021-4028.

Fanta-Feofiloff, E., de Brito Eiras, D. R., Boscardim, A. T., & Lacerda-Krambeck, M. (1986). Effect of salinity on the behavior and oxygen consumption of Mugil curema (Pisces, Mugilidae). *Physiology & behavior*, *36*(6), 1029-1034.

Girvan, J. R., & Braithwaite, V. A. (1998). Population differences in spatial learning in three–spined sticklebacks. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *265*(1399), 913-918.

Griffin, A. S., Guez, D., Federspiel, I., Diquelou, M., & Lermite, F. (2016). 3 Invading New Environments: A Mechanistic Framework Linking Motor Diversity and Cognition to Establishment Success. *Biological Invasions and Animal Behaviour*, 26.

Hayes, K. R., & Barry, S. C. (2008). Are there any consistent predictors of invasion success?. *Biological invasions*, *10*(4), 483-506.

Hohenlohe, P. A., Bassham, S., Etter, P. D., Stiffler, N., Johnson, E. A., & Cresko, W. A. (2010). Population genomics of parallel adaptation in threespine stickleback using sequenced RAD tags. *PLoS genetics*, *6*(2), e1000862.

Holway, D. A., & Suarez, A. V. (1999). Animal behavior: an essential component of invasion biology. *Trends in Ecology & Evolution*, *14*(8), 328-330.

Huntingford, F. A. (1976). The relationship between anti-predator behaviour and aggression among conspecifics in the three-spined stickleback, Gasterosteus aculeatus. *Animal Behaviour*, *24*(2), 245-260.

Kabadayi, C., Bobrowicz, K., & Osvath, M. (2018). The detour paradigm in animal cognition. *Animal cognition*, *21*(1), 21-35.

Kolar, C. S., & Lodge, D. M. (2001). Progress in invasion biology: predicting invaders. *Trends in ecology & evolution*, *16*(4), 199-204.

Lockwood, J. L., Cassey, P., & Blackburn, T. (2005). The role of propagule pressure in explaining species invasions. *Trends in ecology & evolution*, *20*(5), 223-228.

Lockwood, J. L., Cassey, P., & Blackburn, T. M. (2009). The more you introduce the more you get: the role of colonization pressure and propagule pressure in invasion ecology. *Diversity and Distributions*, *15*(5), 904-910.

Lockwood, J. L., Hoopes, M. F., & Marchetti, M. P. (2013). *Invasion ecology*. John Wiley & Sons.

Mettke-Hofmann, C., Lorentzen, S., Schlicht, E., Schneider, J., & Werner, F. (2009). Spatial neophilia and spatial neophobia in resident and migratory warblers (Sylvia). *Ethology*, *115*(5), 482-492.

Mooney, H. A., & Cleland, E. E. (2001). The evolutionary impact of invasive species. *Proceedings of the National Academy of Sciences*, *98*(10), 5446-5451.

Odling-Smee, L., & Braithwaite, V. A. (2003). The influence of habitat stability on landmark use during spatial learning in the three-spined stickleback. *Animal Behaviour*, *65*(4), 701-707.

Peichel, C. L., Ross, J. A., Matson, C. K., Dickson, M., Grimwood, J., Schmutz, J., ... & Kingsley, D. M. (2004). The master sex-determination locus in threespine sticklebacks is on a nascent Y chromosome. *Current biology*, *14*(16), 1416-1424.

R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Sakai, A. K., Allendorf, F. W., Holt, J. S., Lodge, D. M., Molofsky, J., With, K. A., ... & McCauley, D. E. (2001). The population biology of invasive species. *Annual review of ecology and systematics*, *32*(1), 305-332.

Shettleworth, S. J. (2010). *Cognition, evolution, and behavior*. Oxford University Press.

Sih, A., Bolnick, D. I., Luttbeg, B., Orrock, J. L., Peacor, S. D., Pintor, L. M., ... & Vonesh, J. R. (2010). Predator–prey naïveté, antipredator behavior, and the ecology of predator invasions. *Oikos*, *119*(4), 610-621.

Simberloff, D. (2009). The role of propagule pressure in biological invasions. *Annual Review of Ecology, Evolution, and Systematics*, *40*, 81-102.

Stansfield, K. H., & Kirstein, C. L. (2006). Effects of novelty on behavior in the adolescent and adult rat. *Developmental Psychobiology: The Journal of the International Society for Developmental Psychobiology*, *48*(1), 10-15.

Suarez, A. V., & Tsutsui, N. D. (2008). The evolutionary consequences of biological invasions. *Molecular Ecology*, *17*(1), 351-360.

Whitney, K. D., & Gabler, C. A. (2008). Rapid evolution in introduced species, 'invasive traits' and recipient communities: challenges for predicting invasive potential. *Diversity and Distributions*, *14*(4), 569-580.

Williamson, M. H., & Fitter, A. (1996). The characters of successful invaders. *Biological conservation*, *78*(1-2), 163-170.

Wolf, M., Van Doorn, G. S., & Weissing, F. J. (2008). Evolutionary emergence of responsive and unresponsive personalities. *Proceedings of the National Academy of Sciences*, *105*(41), 15825-15830.

Wright, T. F., Eberhard, J. R., Hobson, E. A., Avery, M. L., & Russello, M. A. (2010). Behavioral flexibility and species invasions: the adaptive flexibility hypothesis. *Ethology Ecology & Evolution*, *22*(4), 393-404.

Wund, M. A., Valena, S., Wood, S., & Baker, J. A. (2012). Ancestral plasticity and allometry in threespine stickleback reveal phenotypes associated with derived, freshwater ecotypes. *Biological Journal of the Linnean Society*, *105*(3), 573-583.

Wund, M. A., Baker, J. A., Golub, J. L., & Foster, S. A. (2015). The evolution of antipredator behaviour following relaxed and reversed selection in Alaskan threespine stickleback fish. *Animal behaviour*, *106*, 181-189.

APPENDIX A

SUPPLEMENTARY MATERIALS FOR CHAPTER 1

FIGURE

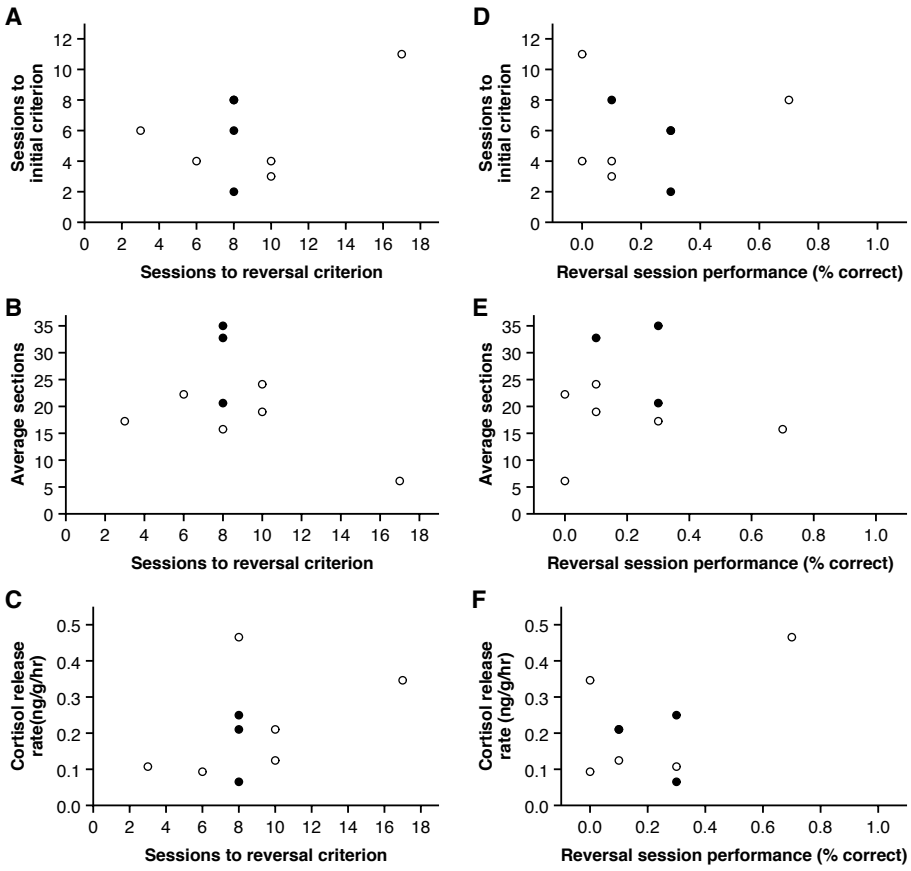


Figure A.1. Scatterplots showing relationships between reversal learning performance (a-c) or flexibility (i.e., proportion correct on first reversal session; d-f) and other traits. Each data point represents a different individual, coded by sex (open circles = females, closed circles = males).

APPENDIX B

SUPPLEMENTARY MATERIALS FOR CHAPTER 2

FIGURE

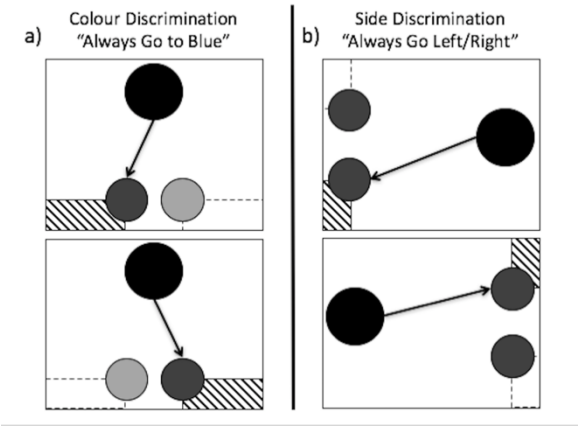


Figure B.1. Top-down view of the tank during training trials for a) color and b) side discrimination learning. Figures are oriented so that the top of each diagram represents the back of the test tank. The large black circle represents the starting shelter and the smaller circles represent search cups (light gray = yellow; dark gray = blue). The arrow point to the correct cup choice. The diagonal stripe region represents the "correct choice" region and the dotted-bordered region represents the "incorrect choice" region.

APPENDIX C

SUPPLEMENTARY MATERIALS FOR CHAPTER 3

TABLE

	β	Std. error	z	p
Initial criterion $^{\delta}$	-0.0423	0.0197	-2.153	0.0313
Reversal criterion $^{\delta}$	-0.0196	0.0112	-1.762	0.0781
Reversal session 1 $^{\delta}$	-0.0382	0.0276	-1.383	0.1666
Reversal trial 1 persist$^{\psi}$	0.0249	0.0580	0.428	0.669
Boldness	0.0642	0.0396	-1.619	0.1116
$PC1_{novel\ object}$	-0.0291	0.0501	-0.580	0.564
$PC1_{barrier}$	9.962e-05	4.435e-02	0.002	0.998

δ = negative binomial family model; ψ=binomial family model

Table C.1. Estimate results of models testing for the effect of length on learning and behavior.